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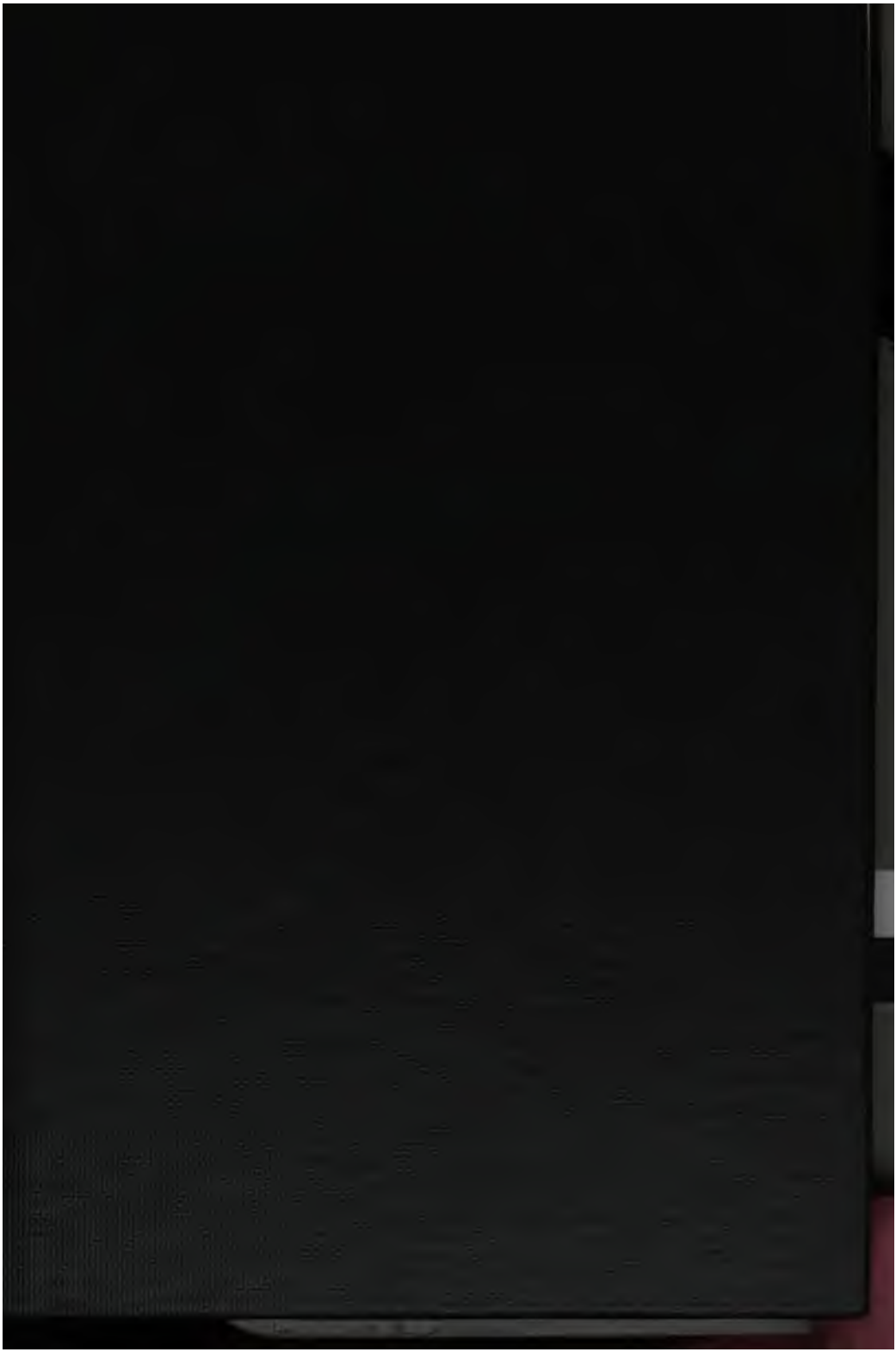

MONOGRAPHS ON EXPERIMENTAL BIOLOGY

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THE NATURE OF ANIMAL LIGHT

BY
E. NEWTON HARVEY, PH.D.

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MONOGRAPHS ON EXPERIMENTAL BIOLOGY

THE NATURE OF ANIMAL LIGHT

BY
E. NEWTON HARVEY, PH.D.
PROFESSOR OF PHYSIOLOGY, PRINCETON UNIVERSITY

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EDITORS' ANNOUNCEMENT

THE rapid increase of specialization makes it impossible for one author to cover satisfactorily the whole field of modern Biology. This situation, which exists in all the sciences, has induced English authors to issue series of monographs in Biochemistry, Physiology, and Physics. A number of American biologists have decided to provide the same opportunity for the study of Experimental Biology.

Biology, which not long ago was purely descriptive and speculative, has begun to adopt the methods of the exact sciences, recognizing that for permanent progress not only experiments are required but quantitative experiments. It will be the purpose of this series of monographs to emphasize and further as much as possible this development of Biology.

Experimental Biology and General Physiology are one and the same science, in method as well as content, since both aim at explaining life from the physico-chemical constitution of living matter. The series of monographs on Experimental Biology will therefore include the field of traditional General Physiology.

JACQUES LOEB,
T. H. MORGAN,
W. J. V. OSTERHOUT.

PREFACE

BIOLUMINESCENCE, the production of light by animals and plants, has always excited the admiration of the layman and the wonder of the scientist. It is not surprising that an enormous literature dealing with the subject has grown up. A large part of this literature, however, is made up merely of reports that a certain animal is luminous, or records of especially brilliant phosphorescence of the sea. Among those who have inquired somewhat more carefully into the nature and causes of light production may be mentioned the names of Beijerinck, R. Boyle, Dahlgren, Dubois, Ehrenberg, Krukenberg, Mangold, McDermott, Molisch, Panceri, Pflüger, Phipson, Quatrefages, Spallanzani, and Trojan. Several of these men have written comprehensive monographs on the subject.

It is not the purpose of this book to deal with every phase of bioluminescence. Volumes could be written on the evolutionary side of the problem and the structure and uses of luminous organs. These questions can only be touched upon. Neither is it my purpose to discuss the ultimate cause of the light, whether due to vibration of electrons or to other causes. That problem must be left to the physicist, although it is highly probable that a study of animal light will give important information regarding the nature of light in general, and no theory of light can be adequate which fails to take into account the extraordinary powers of luminous animals.

We shall be concerned largely with the physical characteristics of animal light and the chemical processes

underlying its production. Great advances have been made since the first early guesses that the light was due to phosphorus and was a kind of oxidation. Although the problem cannot be considered as solved, it has been placed on a sound physico-chemical basis. Some material is oxidized. Exactly what this material is and why light accompanies its oxidation are the two more fundamental problems in the field of Bioluminescence. How far and with what success we have progressed toward a solution of these problems may be seen from a perusal of the following pages.

It gives me pleasure to acknowledge the kindness of Dr. W. E. Forsythe of the Nela Institute, Cleveland, Ohio, in reading and criticizing the manuscript of Chapter III, and of Professor Lyman of Harvard University for a similar review of Chapter II. I am also deeply indebted to my wife for reading the proof and to Dr. Jacques Loeb and Prof. W. J. V. Osterhout for many suggestions throughout the book. My thanks are also due to Prof. C. Ishikawa of the Agricultural College, Imperial University of Tokio, Japan, for his generous assistance in providing *Cypridina* material. Finally I wish to acknowledge the support of the Carnegie Institution of Washington, through its director of Marine Biology, Dr. Alfred G. Mayor. Without this support much of the work described in this book could not have been accomplished.

E. N. H.

PRINCETON, N. J.,
October, 1919.

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THE NATURE OF ANIMAL LIGHT

CHAPTER I

LIGHT-PRODUCING ORGANISMS

THE fact that animals can produce light must have been recognized from the earliest times in countries where fireflies and glowworms abound, but it is only since the perfection of the microscope that the phosphorescence of the sea, the light of damp wood and of dead fish and flesh has been proved to be due to living organisms. Aristotle mentions the light of dead fish and flesh and both Aristotle and Pliny that of damp wood. Robert Boyle in 1667 made many experiments to show that the light from all three sources, as well as that of the glowworm, is dependent upon a plentiful supply of air and drew an interesting comparison between the light of shining wood and that of a glowing coal. Boyle had no means of finding out the true cause of the light and early views of its nature were indeed fantastic. Even as late as 1800 Hulme concludes from his experiments on phosphorescent fish that the light is a "constituent principle of marine fishes" and the "first that escapes after the death of the fish." It was only in 1830 that Michaelis suspected the light of dead fish to be the result of some living thing and in 1854 Heller gave the name *Sarcina noctiluca* to the suspected organism. In 1875 Pflüger showed that nutrient media could be inoculated with small amounts of luminous fish and that these would increase in size, like bacterial

colonies, and we now know that the light of all dead fish and flesh is due to luminous bacteria.

In the early part of the nineteenth century it was surmised that the light of damp wood was connected with fungus growth because of a similarity in smell. In 1854 Heller recognized minute strands, which he called *Rhizomorpha noctiluca,* as the actual source of the light. We now know that all phosphorescent wood is due to the mycelium of various kinds of fungi and that sometimes the fruiting body of the fungus also produces light.

The phosphorescence or "burning of the sea," which is described by so many of the older explorers, is also due entirely to living organisms, both microscopic and macroscopic. The latter are mostly jelly-fish (*medusæ*) or comb jellies (*Ctenophores*) and give rise to the larger, more brilliant flashes of light often seen in the wake or about the sides of a steamer at night. The former are various species of dinoflagellates or cystoflagellates such as *Noctiluca* (just visible to the naked eye) which collect at the surface of the sea and often increase in such numbers that the water is colored by day (usually pink or red) and shines like a sheet of fire when disturbed at night. Although *Noctiluca* was recognized as a luminous animal in 1753 by Baker, the light of the sea was a mysterious phenomenon to the older observers. MacCartney, speaking before the Royal Society in 1810, outlines the various older theories as follows: "Many writers have ascribed the light of the sea to other causes than luminous animals. Martin supposed it to be occasioned by putrefaction; Silberschlag believed it to be phosphoric; Prof. J. Mayer conjectured that the surface of the sea imbibed light, which it afterwards discharged. Bajon and Gentil

thought the light of the sea was electric, because it was excited by friction. . . . I shall not trespass on the time of the Society to refute the above speculations; their authors have left them unsupported by either arguments or experiments, and they are inconsistent with all ascertained facts upon the subject. The remarkable property of emitting light during life is only met amongst animals of the four last classes of modern naturalists, viz., mollusca, insects, worms, and zoöphytes.." MacCartney recognized the true cause of the light, although he had little idea of the vast number of marine forms which are luminous and omits entirely any reference to the fishes, many of which produce a light of their own when living, apart from any bacterial infection.

A survey of the animal kingdom discloses at least 36 orders containing one or more forms known to produce light and several more orders containing species whose luminosity is doubtful. In the plant kingdom there are two groups containing luminous forms. The distribution of luminous organisms is brought out in the accompanying classification of plants and animals. Those orders are printed in italics which contain species whose self-luminosity is fairly well established. It will be noted that further subdivisions into orders is not given in classes of animals which lack luminous forms.

TABLE 1

DISTRIBUTION OF LUMINOUS ORGANISMS IN PLANT AND ANIMAL KINGDOMS

PLANT KINGDOM

I. *Thallophyta*
  Algæ
    Cyanophyceæ (Blue-green Algæ)
    Chlorophyceæ (Green Algæ)

Phæophyceæ (Brown Algæ)
Rhodophyceæ (Red Algæ)
Lichenes (Lichens, symbiotic growth of algæ and fungi)
Fungi
Myxomycetes (Slime moulds)
Schizomycetes (Bacteria)
Bacterium, Photobacterium, Bacillus. Pseudomonas, Micrococcus, Microspira, Vibrio.
Phycomycetes (moulds)
Ascomycetes (Sac fungi, yeasts, some moulds)
Basidiomycetes (Smuts, rusts, mushrooms)
Ustilaginæ (Smuts)
Uridineæ
Auriculariæ (Judas ears)
Tremellineæ (Jelly fungi)
Hymenomycetes (Mushrooms)
Agaricus, Armillaria, Pleurotus, Panus, Mycena, Omphalia, Locellina, Marasinium, Clitocybe, Corticium.
Gasteromycetes (Stinkhorns and puff-balls)

II. Bryophyta
Hepaticæ (Liverworts)
Musci (Mosses)

III. Pteridophyta
Equisetineæ (Horsetails)
Salviniæ (Salvinia, Marsilia, etc.)
Lycopodineæ (Club Mosses)
Filicineæ (Ferns)

IV. Spermatophyta
Gymnospermæ (Cycads, Ginkgo, Conifers)
Angiospermæ (Mono- and Dicotyledonous flowering plants).

ANIMAL KINGDOM

I. *Protozoa.* (One-celled animals)
Sarcodina
Rhizopoda
Heliozoa
Radiolaria
Thallassicola, Myxosphæra, Collosphæra, Collozoum, Sphærozoum.
Mastigophora

Flagellata
Choanoflagellata
Dinoflagellata
Ceratium, Peridinium, Prorocentrum, Pyrodinium, Gonyaulax, Blepharocysta, Amphidinium, Diplopsalis, Cochlodinium, Sphærodinium, Gymnodinium.
Cystoflagellata
Noctiluca, Pyrocystis, Leptodiscus, Craspedotella.
Sporozoa
Infusoria

II. Porifera (Sponges)
Calcarea
Hexactinellida
Desmospongiæ

III. *Cœlenterata*
Hydrozoa (Hydroids and Jelly-fish)
Leptomedusæ or *Campanulariæ*
Medusa form—*Eutima, Phyalidium* (*Oceania*).
Hydroid form—*Aglaophenia, Campanularia, Sertularia, Plumularia, Cellularia, Valkeria, Obelia, Clytia.*
Trachomedusæ
Geryonia, Lyriope, Aglaura . .
Narcomedusæ
Cunina,
Anthomedusæ or *Tubulariæ*
Medusa form—*Thaumantias, Tiara, Turris, Sarsia.*
Hydroid form— ?
Hydrocorallinæ
Siphonophora
Abyla, Praya, Diphyes, Eudoxia, Hippopodius.
Scyphozoa (Jelly-fish)
Stauromedusæ
Peromedusæ
Cubomedusæ
Carybdia
Discomedusæ
Pelagia, Aurelia, Chrysaora, Rhizostoma, Cyanœa, Dianea, Mesonema.
Actinozoa (Corals, Sea-fans, Sea-pens, Sea-anemones)
Actinaria
Madreporareia

Antipatharia
Alcyonaria
Alcyonium, Gorgonia, Isis, Mopsea
Pennatulacea
Pennatula, Pteroides, Veretillum, Cavernularia.
Funicularia, Renilla, Pavonaria, Stylobelemon, Umbellularia, Virgularia?
Ctenophora (Comb-jellies)
Cydippida
Pleurobranchia.
Lobata
Mnemiopsis, Bolinopsis, Leucothea (*Eucharis*).
Cestida
Cestus.
Beroida
Beroë.

IV. Platyhelminthes
Turbellaria (Flat-worms)
Trematodes (Parasitic flat-worms)
Cestodes (Tape-worms)
Nemertinea (Nemertines)

V. Nemathelminthes
Nematoda (Round worms)
Gordiacea (Hair worms)
Acanthocephala (Acanthocephalids)
Chætognatha (Sagitta)

VI. Trochelminthes
Rotifera (Wheel animalcules)
Gastrotricha (Chætonotus))
Kinorhyncha (Echinoderes)

VII. *Molluscoidea*
Bryozoa (Corallines)
Entoprocta
Ectoprocta
Membranipora, Scrupocellaria, Retepora? Flustra?
Brachiopoda (Lamp shells)
Phoronidea (Phoronis)

VIII. *Annulata*
Archiannelida (Primitive worms, including Dinophilus)
Chætopoda (True worms)

Polychæta

Chætopterus, Phyllochætopterus, Telepsaris, Polynoë, Acholoë, Tomopteris, Odontosyllis, Lepidonotus, Pionosyllis, Phyllodoce, Heterocirrus, Polyopthalamus?

Oligochæta

Lumbricus, Photodrilus, Allolobophora (Eisemia), Microscolex, Nonlea, Enchytræus, Octochætus.

Gephyrea (Sipunculus)

Hirudinea (Leeches)

Myzostomida (Myzostomus)

IX. *Echinodermata*

Asteroidea (Star-fish)

Ophiuroidea (Brittle-stars)

Ophiurida

Ophiopsila, Amphiura, Ophiacantha, Ophiothrix, Ophionereis.

Euryalida

Echinoidea (Sea urchins)

Holothuroidea (Sea Cucumbers)

Crinoidea (Feather-stars)

X. *Arthropoda*

Crustacea (Crabs, lobsters, shrimps, etc.)

Phyllapoda

Ostracoda

Halocypris, Cypridina, Pyrocypris, Conchœcia, Cyclopina.

Copepoda

Metridia, Leuckartia, Pleuromma, Oncœa, Heterochæta.

Cirripedia

Phyllocardia

Schizopoda

Nyctiphanes, Nematoscelis, Gnathophausia, Euphausia, Stylochiron, Boreophausia, Mysis?

Decapoda

Sergestes, Aristeus, Heterocarpus, Hoplophorus, Acanthephyra, Pentacheles, Colossendeis

Stomatopoda

Cumacea

Amphipoda

Isopoda

Onychophora (Peripatus)

Myriapoda (Centipedes and Millepedes)

Symphyla

Chilopoda

Geophilus, Scolioplanes, Orya.
Diplopoda
Pauropoda
Insecta (Insects)
Aptera (Spring-tails)
Lipura, Amphorura, Neanura
Orthoptera
Neuroptera
Teleganoides and Cœnis of the Mayflies? *Termites?*
Hemiptera
Diptera (Flies)
Bolitophila and *Ceroplatus* larvæ, *Thyreophora?*
Coleoptera (Beetles)
Pyrophorus, Photophorus, Luciola, Lampyris, Phengodes, Photuris, Photinus, etc.
Lepidoptera
Hymenoptera
Arachnida (Spiders)

XI. *Mollusca*
Amphineura (Chiton)
Pelecypoda (Bivalves)
Protobranchia
Filibranchia
Pseudo-Lamellibranchia
Eu-lamellibranchia
Pholas
Septibranchiata
Gasteropoda (Snails, periwinkles, slugs, etc.)
Prosobranchiata
Ophisthobranchiata
Phyllirrhoë, Plocamopherus.
Pulmonata
Scaphopoda (Dentalium)
Cephalopoda (Squids and Octopus)
Tetrabranchiata
Dibranchiata decapoda
Onychoteuthis, Chaunoteuthis, Lycoteuthis, Nematolampas, Lampadioteuthis, Enoploteuthis, Abralia, Abraliopsis, Watasenia, Ancistrocheirus, Thelidioteuthis, Pterygioteuthis, Pyroteuthis, Octopodoteuthis ?, Calliteuthis, Histioteuthis, Benthoteuthis, Hyaloteuthis, Eucleoteuthis, Chiroteuthis,

Mastigoteuthis, Cranchia, Liocranchia, Pyrgopsis, Leachia, Liguriella, Phasmatopsis. Toxeuma, Megalocranchia, Leucocranchia, Crystalloteuthis, Phasmatoteuthis, Galiteuthis, Corynomma, Henseniotenthis, Bathothauma, Rossia ?, Heteroteuthis, Iridoteuthis, Sepiola, Rondeletia. Inioteuthis, Euprymna, Melanoteuthis?.

XII. *Chordata*

Adelochorda (Balanoglossus)
Balanoglossus, Ptychodera, Glossobalanus
Urochorda (Ascidians)
Larvacea
Appendicularia ?
Thaliacea
Salpa, Doliolum?
Ascidiacea
Pyrosoma, Phallusia
Acrania (Amphioxus)
Cyclostomata (Cylostomes)
Pisces (Fishes)
Elasmobranchii
Centroscyllium, Spinax, Paracentroscyllium, Isistius, Lœmargus, Euproctomicrus, Benthobatis?
Holocephalii
Dipnoi
Teleostomi
Stomias, Chauliodus, Melanostomius, Pachystomias, Bathophilus, Dactylostomius, Malacosteus, Astronesthes, Ophozstomias, Idiacanthus, Bathylychnus, Macrostomius, Gonostoma, Cyclothone, Photichthys, Vinciguerria, Ichthyococcus, Lychnopoles, Diplophos, Triplophos, Valenciennellus, Maurolicus, Argyropelecus, Sternoptyx, Polyipnus, Ipnops? Neoscopelus, Myctophum, Halosausus, Xenodermichthys? Macrurus? Photoblepharon, Anomalops, Porichthys, Leuciocornus, Mixonus? Bassozetus? Oneirodes, Ceratias, Gigantactis, Chaunax, Malthopsis, Halicmetus, Monocentris, Lamprogrammus.
Amphibia (Frogs, Toads, Salamanders)
Reptilia (Snakes, Lizards, Turtles)
Aves (Birds)
Mammalia (Mammals)

The only groups of the plant kingdom which are known to produce light are some of the bacteria and some of the fungi and the dinoflagellates (*Peridineæ*) if one is to include them among the plants. Many different species of phosphorescent bacteria have been described, differing in cultural characteristics and structural peculiarities and grouped in the genera, *Bacterium, Photobacterium, Bacillus, Microspira, Pseudomonas, Micrococcus,* and *Vibrio.* Specific names indicating their light-producing power such as *phosphorescens, phosphoreum, luminosum, lucifera,* etc., have been applied.

All the fungi which are definitely known to produce light belong to the *Basidiomycetes,* the largest and most highly developed of the true fungi. Either the mycelium alone or the fruiting body alone, or both, may be luminescent.

Among animals the best known forms are the dinoflagellates; *Noctiluca;* hydroids; jelly-fish; ctenophores; sea pens; *Chætopterus* and other marine worms; earthworms; brittle stars; various crustaceans; myriapods; fireflies and glowworms, the larvæ of fireflies; *Pholas dactylus* and *Phyllirrhoë bucephala,* both molluscs; squid; *Pyrosoma,* a colonial ascidian; and fishes.

Luminous animals are all either marine or terrestrial forms. No examples of fresh water luminous organisms are known. Of marine forms, the great majority are deep sea animals, and it is among these that the development of true luminous organs of a complicated nature is most pronounced. Many of the luminous marine animals are to be found in the plankton, while the littoral luminous forms are in the minority. Some members of all the above groups are found at one or another of our marine labora-

tories with the possible exception of *Pholas, Phyllirrhoë* and squid. Although earthworms and myriapods which produce light are found in the United States, they are rather rare and seldom observed forms.

Not only adult forms but the embryos and even the eggs of some animals are luminous. The egg of *Lampyris* emits light within the ovary and freshly laid eggs are quite luminous. The light does not come from luminous material of the luminous organ adhering to the egg when it is laid but from within the egg itself. *Pyrophorus* eggs are also luminous. The segmentation stages of *Ctenophores* are luminous on stimulation, as noted by Allman (1862), Agassiz (1874) and Peters (1905), but the eggs themselves do not luminesce. *Schizopod larvæ* (Trojan, 1907), *Copepod nauplii* (Giesbrecht, 1895), *Chætopterus larvæ* (Enders, 1909), and brittle star *plutei* (Mangold, 1907) also produce light.

Apparently there is no rhyme or reason in the distribution of luminescence throughout the plant or animal kingdom. It is as if the various groups had been written on a blackboard and a handful of sand cast over the names. Where each grain of sand strikes, a luminous species appears. The *Cœlenterates* have received most sand. Luminescence is more widespread in this phylum and more characteristic of the group as a whole than any other. Among the arthropods luminous forms crop up here and there in widely unrelated groups. In the mollusks, excluding the cephalopods, only two luminous species are known. Several *phyla* contain no luminous forms whatever. It is an extraordinary fact that one species in a genus may be luminous and another closely allied species contain no trace of luminosity. There seems to have been

no development of luminosity along direct evolutionary lines, although a more or less definite series of gradations with increasing structural complexity may be traced out among the forms with highly developed luminous organs.

While the accompanying list of luminous genera aims to be fairly complete, there are no doubt omissions and some inaccuracies in it. Anyone who has ever tried to determine what animal is responsible for the occasional flashes of light observed on agitating almost any sample of sea water will realize how difficult it is to discover the luminous form among a host of non-luminous ones, especially if the animal is microscopic in size. It is not surprising, then, to find many false reports of luminous animals in the literature of the subject and we cannot be too careful in accepting as luminous a reported case. The difficulty lies chiefly in the fact that all luminous organisms with the exception of bacteria, fungi, and a few fish, flash only on stimulation, and, while it is easy enough to see the flash, the animal is lost between the flashes. The only safe way to detect luminous organisms is to add a little ammonia to the sea water. This slowly kills the organisms and causes any luminous forms to glow with a steady, continuous light for some time, a condition accompanying the death of the animal. Not all observers, however, have followed this method. One must always be on guard against confusing the light from a supposed luminous form with the light from truly luminous organisms living upon it. The reported cases of luminosity among marine algæ are now known to be due to hydroids or unicellular organisms living on the alga.

We know also that many non-luminous forms may become infected with luminous bacteria, not only after

death, but also while living, so that their luminescence is purely secondary. Giard and Billet (1889–90) succeeded in inoculating many different kinds of amphipod crustacea (*Talitrus, Orchestia, Ligia*) and isopod crustacea (*Porcellio, Philoscia*) with luminous bacteria, in some cases passing the infection from one to the next through nine individuals. Curiously enough the bacterium did not produce light on artificial culture media but did when growing in the body of the crustacea, which were killed in about seven days by the infection. The species of *Talitrus* and *Orchestia* might easily have been taken for truly luminous animals if not carefully investigated.

Tarchanoff (1901) has injected luminous bacteria into the dorsal lymph sac of frogs with the result that the animals continued to glow for three to four days, especially about the tongue. I remember once while collecting luminous beetles in Cuba, I was astounded to find a frog which was luminous. Expecting this animal to be of great interest, I examined it further only to find that the frog had just finished a hearty meal of fireflies, whose light was shining through the belly with considerable intensity.

Infection with luminous bacteria is especially liable to occur in any dead marine animal. The flesh is an excellent culture medium. I have seen non-luminous species of squid, recently killed, covered with minute growing colonies, quite evenly spaced, so as to closely resemble luminous species whose light is restricted to scattered light organs over the surface of the body.

Indeed Pierantoni (1918) has carried this idea to extremes. He believes that in the luminous organs of fireflies, cephalopods and *Pyrosoma,* luminous symbiotic

bacteria occur which are responsible for the light of these animals, and he claims in the case of cephalopods and *Pyrosoma* to have been able to isolate these in pure culture on artificial culture media. In the firefly they can be seen but not grown and in luminous animals where no visible bacteria-like structures are apparent he believes we are dealing with ultra-microscopic luminous bacteria similar to the pathogenic forms suspected in filterable viruses. While the assumption of ultra-microscopic organisms makes the refutation of Pierantoni's views a somewhat hazardous task, no one can deny that even an ultra-microscopic organism will be killed by boiling with 20 per cent. (by wt.) HCl for 6 hours. As we shall see, the luminous material of *Cypridina,* an ostracod crustacean, can withstand such prolonged boiling with strong acid. The light of one animal at least, and I believe many others also, cannot be due to any sort of symbiotic organism.

Apart from these cases where light is actually produced but is not primary, not produced by the animal itself, there are many forms whose surface is so constituted as to produce interference colors. This is true in many cases among the birds and butterflies whose feathers and scales are iridescent. Some of these have been erroneously described as luminous. Perhaps the best known case among aquatic animals is *Sapphirina,* a marine copepod living at the surface of the sea, and especially likely to be collected with other luminous forms. Its cuticle is so ruled with fine lines as to diffract the light and flash on moving much as a fire opal. Needless to say no trace of light is given off from this animal in a totally dark room.

It has often been supposed that the eye of a cat or of other animals is luminous. The eyes of a moth, also,

can be seen to glow like beads of fire when it is flying about a flame. Both of these cases are, however, purely reflection phenomena and due to reflection out of the eye again of light which has entered from some external source. The correct explanation was given by Prevost in 1810. The eye of any animal is quite invisible in absolute darkness. The same explanation applies to the moss, *Schistostega,* which lives in dimly illuminated places and whose cells are almost spherical, constructed like a lens, so as to refract the light and condense it on the chloroplasts at the bottom of the cells. Some of this light is reflected out of the cells again and gives the appearance of self-luminosity. The alga, *Chromophyton rosanoffii,* is another example of apparent luminosity, due to reflection from almost spherical cells.

There are several light phenomena known which have nothing to do with living organisms. Commonest of these is St. Elmo's fire ("corposants" of English sailors), a glow accompanying a slow brush discharge of electricity, which appears as a tip of light on masts of ships, spires of churches or even the fingers of the hand. It is best seen in winter during and after snowstorms and is a purely electrical phenomenon.

Less well known is th *Ignis fatuus* (Will-o'-the-Wisp, Jack-o'-Lantern, spunkie), a fire seen over marshes and stagnant pools, appearing as a pale bluish flame which may be fixed or move, steady or intermittent. So uncommon is this phenomenon that its nature is not well understood, but it is believed to be the result of burning *phosphine* ($PH_3 + P_2H_4$), a self-inflammable gas, generated in some way from the decomposition of organic matter in the swamp. The difficulty with this explanation is that

phosphine is not known as a decomposition product of organized matter. Methane (CH_4), a well-known decomposition product of organic matter and abundantly formed in swamps, will burn with a pale bluish flame and some have thought the *Ignis fatuus* to be the result of this gas. As methane is not self-inflammable there remains the difficulty of explaining how it becomes lighted. Although still a mystery, it is possible that this light is also of electrical origin or that in some cases large clusters of luminous fungi have been observed.

The flashing of flowers, especially those of a red or orange color, like the poppy, which many observers have noticed during twilight hours, is a purely subjective phenomenon due to the formation of after images in eyes partially adapted to the dark. This flashing, first observed by the daughter of Linnæus, is never observed in total darkness or in the direct field of vision, but only in the indirect field as during a sidelong glance at the plant.

There are some cases of luminosity on record in connection with man himself. (See Heller, 1854). Before the days of aseptic and antiseptic surgery, wounds frequently became infected with luminous bacteria and glowed at night. The older surgeons even supposed that luminous wounds were more apt to heal properly than non-luminous ones. We know that luminous bacteria are non-pathogenic, harmless organisms and the presence of these forms even on dead fish or flesh never accompanies but always precedes putrefaction. As recorded by Robert Boyle, no harm has come from eating luminous meat, unless it may also have become infected with pathogenic forms.

A few cases of luminous individuals have been noted

in which the skin was the source of light, especially if the person sweated freely. It is possible that here we are again dealing with luminous bacteria upon the accumulations of substances passed out in the sweat, which serves as a nutrient medium.

There are also on record, in the older literature, cases of luminous urine, where the urine when freshly voided was luminous. If these observations are correct and they may, perhaps, be doubted, we are at present uncertain of the cause of the light. Bacterial infections of the bladder are not inconceivable although luminous bacteria are strongly aerobic and would not thrive under anaerobic conditions. I can state from my own experiments that luminous bacteria will live in normal human urine, but not well. In albuminous urines it is very likely that they would live better, and it is possible that the luminous urines reported are the results of luminous bacterial infection. On the other hand, the light may be purely chemical, due to the oxidation of some compound, an abnormal incompletely oxidized product of metabolism, which oxidizes spontaneously in the air. We know that sometimes these errors in metabolism occur, as in *alkaptonuria,* where homogentistic acid is excreted in the urine and on contact with the air quickly oxidizes to a dark brown substance. Light, however, has never been reported to accompany the oxidation of homogentistic acid, although it does accompany the oxidation of some other organic compounds. (See Chapter II.)

Finally, we may inquire to what extent luminous animals may be utilized by man. Leaving out of account the use of tropical fireflies for adornment by the natives of the West Indies and South America and the use for bait,

in fishing, of the luminous organ of a fish, *Photoblepharon,* by the Banda islanders, we find that luminous bacteria are of value for certain purposes in the laboratory.

These methods are all due to Beijerinck (1889, 1902). He has, for instance, used luminous bacteria for testing bacterial filters. If there is a crack in the filter the bacteria will pass through and a luminous filtrate is the result, but a perfect filter allows no organisms to pass and gives a dark filtrate.

Luminous bacteria are also very sensitive to oxygen and cease to luminesce in its absence. By mixing luminous bacteria with an emulsion of chloroplasts (from clover leaves) in the dark, allowing the bacteria to use up all the oxygen, and then exposing the mixture to light of various colors, the effect of different wave-lengths in causing photosynthesis could be studied. Only if the chloroplasts are exposed to a color in the spectrum which decomposes CO_2 with liberation of oxygen do the bacteria luminesce, and when this oxygen is used up by the bacteria, the tube again becomes dark. Beijerinck has also worked out a method of testing for maltose and diastase with luminous bacteria, based on the fact that a certain form, *Photobacterium phosphorescens,* will only produce light in presence of maltose or diastase which will form maltose from starch.

Although Dubois and Molisch have both prepared "bacterial lamps" and although it has been suggested that this method of illumination might be of value in powder magazines where any sort of flame is too dangerous, it seems doubtful, to say the least, whether luminous bacteria can ever be used for illumination. Other forms, perhaps, might be utilized, but bacteria produce too weak

a light for any practical purposes. The history of Science teaches that it is well never to say that anything is impossible. It is very unlikely that any luminous animal can be utilized for practical illumination, but there is no reason why we cannot learn the method of the firefly. Then we may, perhaps, go one step further and develop a really efficient light along similar lines. To what extent our inquiry into the "secret of the firefly" has been successful may be gleaned from the following pages.

CHAPTER II

LUMINESCENCE AND INCANDESCENCE

MODERN physical theory supposes that light is a succession of wave pulses in the ether caused by vibrating electrons. The light to which we are most accustomed—sunlight, electric light, gaslight, etc.,—is due to electrical phenomena connected more or less directly with the high temperature of the source of the light. Every solid body above the temperature of absolute zero is giving off waves of different wave-length (λ) and frequency (ν) but of the same velocity (v), in vacuo, 180,000 miles, or 300,000 kilometres a second. In fact, v (a constant)$= \lambda\nu$, so that it is only necessary to designate the wave-length in order to characterize the waves. This is radiant energy or radiant flux.

As everyone knows, the long waves given off in largest amount from objects at comparatively low temperatures give the sensation of warmth. As we raise the temperature, in addition to these longer heat waves, those of shorter and shorter wave-length are given off in sufficient quantity to be detected. At 525° C., rays of about $\lambda = .76\mu$ in length are just visible as a faint red glow to the eye. As the temperature increases still shorter wave-lengths become apparent, and the light changes to dark red (700°), cherry red (900°), dark yellow (1100°), bright yellow (1200°), white-hot (1300°) and blue-white (1400° and above). Above $\lambda = .4\mu$ the waves again fail to affect our eye, and, although they are very active in producing chemical changes, we have no sense organs for perceiving

them. Thus, a white-hot object liberates radiant energy or flux of many different wave-lengths corresponding to what we know as "heat, light and actinic rays." All can be dispersed by prisms of one or another appropriate material to form a wide continuous spectrum, such as that indicated in Fig. 1. Radiant energy of $\lambda = .76\mu$ to $\lambda = .4\mu$, evaluated according to its capacity to produce the sensation of light, is spoken of as visible radiation or luminous flux.

Below the infra-red comes a region of wave-length as yet uninvestigated, and beyond this may be placed the Hertzian electric waves of long wave-length used in wireless telegraphy. Above the ultra-violet comes another region as yet uninvestigated, and then Röntgen rays (X-rays) and radium rays, of exceedingly short wave-length. These last types need not concern us except in that we may later inquire if they are given off by luminous animals. The shortest of the ultra-violet are known as Schumann and Lyman rays. These relations are brought out in Table 2.

TABLE 2.

Wave-lengths of Various Kinds of Radiation

Wave-lengths of light are usually given in Ångstrom units. One micron (μ) = .001 mm. = 1000 millimicrons ($\mu\mu$) = 10,000 Ångstrom units (Å) or tenth metres = 10^{-10} metres or 10^{-8} centimetres. The entire scale of wave-lengths extends from 10^{6} to 10^{-9} centimetres.

Hertzian electric waves (upper limit not reached) above	12 km. to 16 cm.
Unexplored region	.16 cm. to 310μ
Infra-red	310μ to .76μ
Visible light	7600 Å to 4000 Å
Ultra-violet	4000 Å to 320 Å
Unexplored region	320Å to 12 Å
X-rays	12 Å to 0.2 Å
Radium γ rays	0.2 Å and shorter

The total radiant energy which a body emits is a function of its temperature and for a perfect radiator, or what is known as a black body, the total radiation varies as the fourth power of the absolute temperature, T. (Stefan-Boltzmann Law). The radiant energy emitted at different wave-lengths is not the same but more energy is emitted at one particular wave-length ($\lambda_{max.}$) than at longer or shorter ones, depending also on the temperature. If the various waves are intercepted in some way, their relative energy can be measured by an appropriate instrument and spectral energy curves can be drawn, showing the

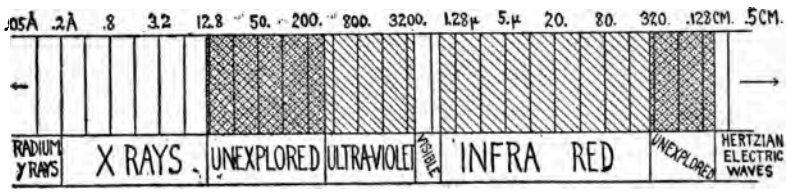


FIG. 1.—Schematic representation of various types of radiation to form a wide continuous spectrum.

distribution of energy throughout the spectrum. Fig. 2 gives a few of the curves, and it will be noted that the maximum shifts toward the shorter waves the higher the temperature. In fact, for a black body $\lambda_{max.} \times T = 2890$, and at 5000° C. (about the temperature of the sun) $\lambda_{max.}$ lies within the visible spectrum. In gas or electric lights it lies in the infra-red region. The area enclosed by these spectral energy curves represents the total energy emitted, and, knowing this and the area enclosed by the curve of visible radiation, it is easy to determine how efficient a source of light is as a light-producing body. We shall inquire more fully into this question in Chapter III, in considering the efficiency of the firefly as a source of light.

A body which emits light because of its (high) temperature is said to be incandescent and we speak of temperature radiation. We know, however, of many cases where substances give off light at temperatures much below 525° C. They do not follow the Stefan-Boltzmann law. The light emission is stimulated by some other means than heat. Such bodies we speak of as *luminescent,* and in this category belong all luminous animals. The distinction between light and luminescence was first

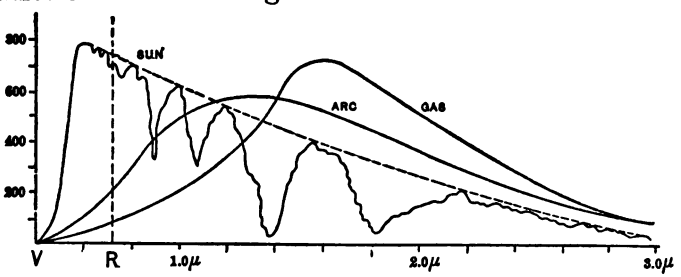


FIG. 2.—Distribution of energy throughout the spectrum of the sun, electric arc, and gas light (*after Nichols and Franklin*). Ordinates show the relative intensities of different wave-lengths emitted. The notches in the curve represent absorption bands and the dotted line represents what the radiation from the sun would be if no selective absorption occurred. V=violet and R=red end of visible spectrum. (*Courtesy Macmillan Co.*)

pointed out by Wiedemann (1888). It is usual to classify luminescences, according to the means of exciting the light, into the following groups:

Thermoluminescence
Phosphorescence and Fluorescence
    Photoluminescence
    Cathodoluminescence
    Anodoluminescence
    Radioluminescence
Triboluminescence and Piezoluminescence
Crystalloluminescence
Chemiluminescence

The luminescence which appears in a vacuum tube when an electric current is passed through it is sometimes spoken of as *electroluminescence.* As electroluminescence and also thermoluminescence are really special cases of phosphorescence or fluorescence and tribo- and crystallo-luminescence are closely allied, the classification has only the merit of emphasizing the means of producing light. Let us examine each kind in turn in order that we may place the light of animals, *organoluminescence* or *bioluminescence* (or *biophotogenesis*), in one of these classes. All are examples of "cold light," light produced at temperature far below those observed in incandescent solids. In this category should be placed also the light from salts in the bunsen flame, for flame spectra and line spectra in general, while only obtained at relatively high temperatures, are not to be confused with the purely temperature radiation from the incandescent particles of carbon in a gas or candle light. The sodium or lithium flame, etc., is not a simple function of temperature and has been spoken of as a luminescence, *pyroluminescence.* As the luminescence of organisms could in no manner be regarded as a pyroluminescence, occurring at temperatures far above those compatible with life, a consideration of this form of luminescence will be omitted. Some other low temperature flames are known, such as that of CS_2 in air, rich in ultra-violet rays, despite its relatively low temperature. While these are of interest to the physicist and chemist, they can have no direct bearing on the luminescence of animals and their consideration will also be omitted. (See Bancroft and Weiser, 1914–1915.)

THERMOLUMINESCENCE.—Some substances begin to emit light of shorter wave-length than red, well below 525°.

This is thermoluminescence. Diamond, marble, and fluorite are examples. Only certain varieties of fluorite show the phenomenon well. A crystal of one of these varieties heated in the bunsen flame on an iron spoon will give off a white light long before any trace of redness appears in the iron. Other crystals may luminesce in hot water. In all, this luminescence is dependent on a previous illumination or radiation of the crystal. If kept in the dark for a long time no trace of light appears when fluorite is placed at a temperature of 100°, but after a short exposure to the light of an incandescent bulb, although no light can be observed in the fluorite at room temperature, quite a bright glow appears at 100°. Calcium, barium, strontium, magnesium and other sulphates containing traces of manganese sulphate, show a similar phenomenon after exposure to cathode rays (Wiedemann and Schmidt, 1895 *b*). They emit light during bombardment, but this soon ceases when the rays are cut off. If the sulphates are now heated they give off light, red in the case of $MgSO_4 + MnSO_4$, green in the case of $CaSO_4 + MnSO_4$. The power to emit light on heating may be retained for months after the exposure to cathode rays. The emission of light by bodies after previous illumination or radiation is called *phosphorescence* and will be considered below. It would seem that the cases of thermoluminescence with which we are acquainted are really cases of phosphorescence intensified by rise of temperature. The spectrum of thermoluminescent bodies, also, is similar to that of phosphorescent ones. (See Fig. 3.) However, not all phosphorescent materials are also thermoluminescent. The production of light by animals is quite another phenomenon from thermoluminescence.

PHOSPHORESCENCE AND FLUORESCENCE.—Although the word phosphorescence has been used in a very loose way to indicate all kinds of luminescence, and particularly that of phosphorus or of luminous animals, to the physicist it has a very definite meaning, namely, the absorption of radiant energy by substances which afterwards give this off as light. Phosphorescence does not strictly apply to the light of white phosphorus. If the radiant energy is light (visible or ultra-violet) we speak of *photoluminescence,* if cathode rays we have *cathodoluminescence,* if anode rays, *anodoluminescence,* and if X-rays (Röntgen rays) we have *radioluminescence.* Inasmuch as the α, β, and γ rays of radium correspond to the anode, cathode, and X-rays, respectively, radium radiation also produces luminescence in many kinds of material. If the material gives off the light only during the time it is radiated we speak of fluorescence; if the light persists we speak of phosphorescence. The distinction is perhaps a purely arbitrary one, as there are a great many substances which give off light for only a fraction of a second (1/5000 sec. in some cases) after being illuminated (*photoluminescence.*) Some substances also, which fluoresce at ordinary temperatures, will phosphoresce at low temperatures. Phosphorescence is exhibited chiefly by solids, fluorescence also by liquids and vapors.

Special means must be used to observe a phosphorescence of short duration. E. Becquerel has devised an apparatus for doing this, a *phosphoroscope.* It consists of revolving disks with holes in them between which the object to be examined is placed. The holes are so arranged that the object is first illuminated and then completely cut off from light. The observer looking at it through

another hole sees it at the moment it is not illuminated and can thus tell if it is phosphorescing. By determining the rate of revolution of the disks it is easy to calculate how long the phosphorescence persists.

While relatively few solids phosphoresce after exposure to light at ordinary temperature a large number of these acquire the property at the temperature of liquid air. Included in the list are such biological products as urea, salicylic acid, starch, glue and egg shells. The temperature also affects the wave-length and hence the color of the light given off. Usually the higher the temperature the shorter the wave-length, but in the case of some bodies (SrS) the wave-lengths become longer at the higher temperature.

The best known cases of phosphorescence which occur at room temperature and the group to which the word phosphorescence is commonly applied, are those of the alkaline earth sulphides (BaS, CaS, SrS) and ZnS. An Italian, Vicenzo Cascariolo, is said to have discovered the Bologna stone ($BaSO_4$) which, by calcination with charcoal, gave an impure phosphorescent BaS or *lapis solaris*. Canton's phosphorus (CaS) was later prepared "by heating a mixture of three parts of sifted calcined oyster shells with one part of sulphur to an intense heat for one hour." Hulme spoke of it as the "light magnet of Canton," because of its power of attracting and absorbing light. The pure sulphides do not show this property. Only if small amounts of some other metal such as Cu, Pb, Ag, Zn, Sb, Ni, Bi, or Mn are present, will the sulphide phosphoresce. One part of impurity in a million is often sufficient. Such mixtures, together with a flux of Na_2SO_4, $Li_3(PO_4)_2$ or some other fusible salt constitute a "phos-

phor." A "phosphor" is in reality an example of a solid solution and is the basis of some kinds of luminous paints.

The intensity and duration of a phosphorescent light depend chiefly on the nature of the exciting rays, the color chiefly on the impurity present but the alkaline earth metal also exerts an influence. Rise in temperature increases the intensity but diminishes the duration, so that the total amount of light emitted is about constant at different temperatures.

The spectrum of most phosphorescent substances is made up of one or more continuous bands having *maxima* at different wave-lengths. In the light incident on a phosphorescent substance are also bands of light rays which are absorbed and whose wave-lengths are more efficient than others in stimulating phosphorescence. These bands in the phosphorescent light are usually of longer wave-length than those in the light which excites the phosphorescence. This fact is known as Stokes' Law, but it has been found not to be universally true. Curiously enough, red and infra-red rays have the power of annulling phosphorescence after a momentary increase in brightness and phosphorescing materials have been used to determine if infra-red rays are given off in the light of the firefly. Ives (1910) showed that infra-red radiation had no power of quenching the light of the firefly as it does the phosphorescent light of Sidot blende (ZnS), one fact tending to show that the firefly's light is not due to phosphorescence. Fig. 3 is a reproduction of a photograph of the phosphorescence spectrum of ZnS.

Other facts show that the light of luminous animals is in no sense a phosphorescence and is quite independent of previous illumination of the animal. Luminous bac-

teria will continue to luminesce although they are grown in the dark for many weeks. Indeed strong light has a bactericidal action on these forms similar to that with ordinary bacteria. With some marine forms light has an inhibiting effect. They lose their power of luminescence during the day and only regain it at dusk or when kept in the dark for some time. Indeed, ordinary light never has the effect of causing luminescence in the same sense as it causes phosphorescence of CaS.

Fluorescence is most efficiently excited by the cathode rays of a vacuum tube. They not only cause the residual gas in the tube to glow (*electroluminescence*) by which their path may be followed with the eye, but also a vivid fluorescence of the glass walls of the tube, yellow green with sodium glass, blue green with lead and lithium glass. $LiCl_2$ in the path of cathode rays gives off a blue light; in the path of anode rays a red light; NaCl a blue cathodoluminescence and a yellow anodoluminescence. The spectrum of the latter is a line spectrum of Li or Na, showing the characteristic red or yellow lines similar to those observed where Li or Na is held in the bunsen flame. The spectrum of the salts under excitation of cathode rays is a short continuous one in the blue region. Fluorescent spectra in general are of this nature, made up of short bands of light in one or more regions.

Diamonds, rubies and many minerals fluoresce brilliantly in the path of cathode rays. Some specimens of fluorite (CaF_2) show the phenomenon especially well, whence the name *fluorescence.* Fluorescent screens of barium platino-cyanide, willemite (Zn_2SiO_4) Sidot blend (ZnS) or Scheelite (Ca tungstate) are frequently employed to render visible X-rays. The luminous paint most

used at the present time is ZnS containing a trace of radium salt. The rays of the radium continually emitted cause a steady fluorescence of the ZnS. Indeed, if one examines the paint on the hands of a watch with a lens the flash of light from the impact of alpha particles on the ZnS can be distinctly seen, as in the *spinthariscope.*

Some animal tissues and fluids, especially the lens of the eye, will luminesce in the path of radium rays, as shown by the experiments of Exner (1903), but there is no evidence that luminous animals are especially active in this respect. Ultra-violet rays have the same action.

The luminous material of practically all luminous forms, if dessicated sufficiently rapidly, can be obtained in the form of a dry powder which will give off light when moistened with water. Coblentz (1912) has exposed this dry material to light, to the ultra-violet spark, and to X-rays and in no case has a phosphorescence or fluorescence ever been observed. I have examined the action of radium upon *Cypridina* light. There was no intensifying or diminishing effect of twenty milligrams of radium (probably the bromide) on a luminous solution of *Cypridina* material, nor was phosphorescence or fluorescence excited in a non-luminous extract of the animal. We must conclude that animal light is not a fluorescence of any substance due to radiation produced by the animals themselves.

Many solutions show fluorescence in strong lights. This is especially marked in quinine sulphate, mineral oils, eosin, fluorescein, esculin, rhodamin, chlorophyll, etc. The fluorescence of eosin in 10^{-8} grams per cubic centimetre is visible in daylight and 10^{-15} grams per cubic centimetre in the beam from an arc lamp. It is difficult to realize that the

bluish fluorescence of quinine sulphate is really an emission rather than a reflection of light. But a test tube of quinine sulphate solution held in the ultra-violet region of a spectrum will glow with a pale blue light, although it is not illuminated with any rays that are visible to our eyes. Concerning this, Stokes, to whom the word fluorescence and much of our knowledge of the subject is due, says, "It was certainly a curious sight to see the tube" (containing quinine sulphate solution) "instantaneously lighted up when plunged into the invisible rays; it was literally 'darkness visible.'" Quinine sulphate absorbs the ultra-violet converting these rays into visible blue ones. Its spectrum is a short continuous one. Most fluorescent substances convert short into longer wave-lengths (Stokes' Law), but some may cause the reverse change.

A substance, fluorescent in solution, has been found in a few luminous animals, notably in several species of fireflies and also in a non-luminous beetle. It is called *pyrophorine* or *luciferesceine*. Dubois (1886) has ascribed to pyrophorine the power of absorbing invisible rays and transforming them into visible ones, thus increasing the animal's light. That this is not the case has been shown by the work of Coblentz (1909). He photographed the spectrum of the firefly's light and the fluorescent spectrum of luciferesceine. The latter is almost complementary to the former (see Fig. 4) and no trace of the fluorescent spectrum appears in the spectrum of the light of the firefly. McDermott (1911 *a*) has studied the properties of luciferesceine and regards it merely as an incidental material found in many animals of the *Lampyridæ* (in some non-luminous forms) and having no connection with

rubbing or shaking the animals, it is in reality fundamentally different since it is dependent on the presence of oxygen whereas triboluminescence is not.

CRYSTALLOLUMINESCENCE. — Crystalloluminescence is observed when solutions crystallize. It was described by Bandrowski (1894, 1895) in arsenious oxide, in NaF, or if HCl or alcohol is added to hot saturated NaCl solution. A bluish light with sparkling points appeared. All well authenticated cases are exhibited by simple inorganic salts and these are also all triboluminescent. The reverse is not true, however; many triboluminescent substances are not crystalloluminescent. Crystalloluminescence is much less widespread than triboluminescence. Trautz (1905) has studied the matter in a number of compounds and comes to the conclusion that the light is really a special case of triboluminescence in which the growth of individual crystals causes them to rub together. The light becomes much brighter on stirring a mass of crystals which exhibit crystalloluminescence. While in some cases crystalloluminescence is unquestionably due to the triboluminescence of crystals rubbing against each other it is not in every case, as has been clearly shown by the work of Weiser (1918 *b*). He studied luminescence of saturated aqueous alkali halide solutions (NaCl, KCl, etc.,) upon addition of alcohol or of HCl. The salt crystallizes out under these conditions and Weiser found that the light is brightest when the conditions of concentration of alcohol or of HCl are such as to cause heaping up of Na and Cl ions. He believes that the bluish light which appears is due to the combination of ions in the reaction, $Na^+ + Cl^- = NaCl$. Only if this proceeds rapidly enough does luminescence occur. Weiser studied also the crystallo-

luminescence and triboluminescence of $AsCl_3$ and of K_2SO_4. By photographing the luminescence through color screens of different absorptive power (Weiser, 1918, *a*) a spectrum of the light could be obtained, and it was found to be identical in both the tribo- and crystalloluminescent light; in the case of $AsCl_3$, a band in the green-blue, blue and violet. Weiser believes the light in this case also to come from recombination of the ions, $As^{+++} + 3Cl^- = AsCl_3$, and that crystalloluminescence in general is due to rapid reformation of molecules from ions broken up by electrolytic dissociation while triboluminescence is due to rapid reformation of molecules from ions broken up by violent disruption of the crystal. Of course in triboluminescent organic crystals which do not dissociate into ions, some other reaction must be responsible for the light. One thing seems certain, that the two types of luminescence are similar. As Bigelow * remarks, "It is altogether probable that the cause of this (crystalloluminescence) "whatever it may be, is the same as the cause of triboluminescence, whatever that may be."

Crystals are not found in the luminous organs of animals with the exception of the fireflies. In these a layer of cells occurs (see Chapter IV) filled with minute crystals of one of the purine bodies (xanthin or uric acid). One might surmise that the light of the animal was a crystalloluminescence accompanying the formation of these crystals. It is easy to show, however, that the light comes not from the crystal layer but from another layer of cells containing large granules. It is also dependent on the presence of oxygen while crystalloluminescence takes place in the absence of oxygen. The crystal layer possi-

. Theoretical and Physical Chemistry, 1912, p. 516.

bly serves as a reflector. Its significance will be discussed in a later chapter.

The light of luminous organisms is quite generally associated with granules. In one of the centipedes (*Orya barbarica*), which produces a luminous secretion, Dubois

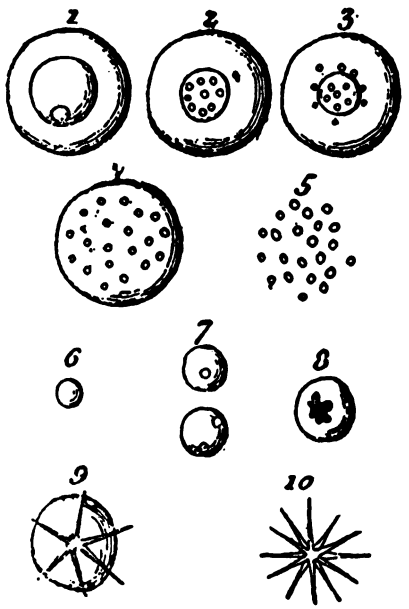


FIG. 5.—Dubois's figures showing transformation of photogenic granules to crystals (*after Dubois*).

(1893) has described the transformation of these granules into crystals and at one time he supposed the light to be a crystalloluminescence. He later reversed this opinion and, certainly, examination of his drawings which are reproduced in Fig. 5 does not convince one of the actuality of crystal formation.

The phenomenon of *lyoluminescence,* described by

Wiedemann and Schmidt (1895) as a light accompanying the solution of colored (from exposure to cathode rays) crystals of Li, Na, or K chlorides, is probably due to a triboluminescence from stirring of the crystals during solution.

CHEMILUMINESCENCE.—As the name implies, chemiluminescence is the production of light during a chemical reaction at low temperatures. This does not mean that the other types of luminescence are not connected with chemical reactions—using the word *reaction* in a broad sense—for we have reason to believe that in some cases spectra are not characteristic of the element as such but are rather characteristic of a particular reaction in which the element takes part (dissociation into ions, changes from monovalent to bivalent condition, etc.) and that this is the reason one element may show various spectra under different conditions (Bancroft, 1913). The chemiluminescences are rather oxidation reactions involving the absorption of gaseous or dissolved oxygen and may be very easily distinguished from all the previously mentioned luminescences by this criterion. They should, perhaps, more properly be called *oxyluminescences.*

The glow of phosphorus is the best known case, recognized since phosphorus was first prepared by Brandt in 1669. It is interesting to note that when first prepared phosphorus was regarded as a peculiarly persistent type of phosphor, *i.e.,* a material akin to the impure alkaline earth sulphides.

Fresh cut surfaces of Na and K metal will glow in the dark for some time, especially if warmed to 60°–70° (Linnemann, 1858). A film of oxide is formed over the surface, showing definitely that oxidation has occurred.

Ozone oxidizes organic matter with an accompanying glow (Fahrig, 1890; Otto, 1896). The light from ozone acting on pyrogallol solution is especially bright under certain conditions.

Radziszewski (1877, 1880) gives a long list of substances, chiefly essential oils, which luminesce if slowly oxidized in alcoholic solutions of alkalis. Formaldehyde, dioxymethylen, paraldehyde, metaldehyde, acroleïn, disacryl, aldehydeammonia, acrylammonia, hydrobenzamid, lophin, hydroanisamid, anisidin, hydrocuminamid, hydrocinamid, besides waxes, and such biological substances as glucose, lecithin, cholesterin, cholic, taurocholic, and glycocholic acids, and cerebrin, all luminesce on oxidation. Radziszewski himself and many other authors have compared the light of organisms to this type of luminescence. Indeed the incorrect identification of granules found in the cells of practically all luminous tissues as oil droplets, is largely due to the influence of Radziszewski's work. Dubois (1901 *b*) has added esculin, and Trautz (1904–5) many aldehydes and phenol derivatives, including vanillin, papaverin, tannic and gallic acids, besides glycerol and mannite to the list of biological substances oxidizing with light production. Guinchant (1905) has described oxyluminescence of uric acid and asparagine, Weitlaner (1911) of substances in humus and McDermott (1913) of substances in urine and the anaerobic alkaline hydrolysis products of glue and Witte's peptone. Pyrogallol is especially prone to luminesce, as was first noticed by Lenard and Wolf (1888) in developing a photographic plate with pyrogallol developer. Later the luminescence was studied in some detail by Trautz and Schloringin (1904–5) who developed the well-known luminescent mix-

ture of pyrogallol, formaldehyde, K_2CO_3 and H_2O_2. As I have shown, pyrogallol can be oxidized in a great many different ways, and some of these are of great interest, for they very closely imitate the mechanism for the production of light in organisms. These are recorded in Table 3, which also includes various other types of oxyluminescence of general or biological interest.

TABLE 3

Types of Oxyluminescent Reactions

1. Oxidation in air spontaneously.
   (*a*) At ordinary temperatures. [Phosphorus. Fresh-cut surfaces of Na or K. Thiophosgene and Thio-ethers (RCS.OR).]
   (*b*) At melting or vaporizing points. (Fats, terpenes, sugars, resins, gums, ether, silk and others.)
2. Oxidation in aqueous or alcoholic alkalies. (Many organic substances.)
3. Oxidation in hypoiodites, hypobromites, or hypochlorites. (Many organic substances.)
4. Oxidation in peroxides (H_2O_2 or Na_2O_2). (Many organic substances.)
5. Oxidation in ozone. (Many organic substances.)
6. Oxidation in acid permanganate. (Pyrogallol.)
7. Oxidation in persulfates and perborates. (Formaldehyde, paraformaldehyde.)
8. Oxidation in perchlorates, periodates, and perbromates. (Palmitic acid.)
9. Combination of 2 and 4. (Many organic substances.)
10. Combination of 3 and 4. (Many organic substances.)
11. Oxidation with H_2O_2 and hæmoglobin or vegetable oxidases. (Pyrogallol, gallic acid, lophin, esculin.)
12. Oxidation with H_2O_2 and MnO_2, $Fe_2Fe(CN)_6$, $Mn(OH)_2 + Mn(OH)_3$, Ag_2O, chromium oxide, cobalt oxide. (Pyrogallol.)
13. Oxidation with H_2O_2 and ferrocyanides, chromates, bichromates, permanganates, Fe salts, and Cr salts. (Pyrogallol, esculin.)
14. Oxidation with H_2O_2 and collodial Ag. Pt. Pd. Au. (Pyrogallol.)

The spectrum of chemiluminescent reactions has been described in a few instances as continuous but no definite measurements of its extent have been made. Radziszew-

ski (1880) found the light of lophin oxidized in alcoholic caustic alkali, examined with a two-prism spectroscope, to give a continuous spectrum, brightest at *E*, with the red and violet ends lacking. Trautz (1905, p. 101) states that the pyrogallol-formaldehyde–Na^2CO_3–H_2O_2 reaction gives a continuous spectrum from the red to the blue green with maximum brightness in the orange. Weiser (1918 *a*) has studied the spectra of some chemiluminescent reactions by photographing the light behind a series of color screens. He finds also that the spectra are short, with maximum intensity in various regions. Thus, *amarin* oxidized by chlorine or bromine, extends from the yellow to greenish blue with a maximum in the green while *phosphorus,* dissolved in glacial acetic acid and oxidized with H_2O_2, luminesces from yellow green to violet.

The spectra of luminous animals are quite similar to those of chemiluminescent reactions. Moreover, as we have seen, chemiluminescence is essentially an oxyluminescence, since oxygen is necessary for the reaction. All luminous animals also require oxygen for light production. Therefore, bioluminescence and chemiluminescence are similar phenomena and they differ from all the other forms of luminescence which we have considered. The light from luminous animals is due to the oxidation of some substance produced in their cells, and when we can write the structural formula of this photogenic substance and tell how the oxidation proceeds, the problem of light production in animals will be solved.

CHAPTER III

PHYSICAL NATURE OF ANIMAL LIGHT

INTEREST in the light of animals from a physical standpoint has centred around questions of quality, efficiency and intensity, but in only one group of luminous animals, the beetles, have accurate measurements of these characteristics been made. This is due in part to the abundance of these forms and their appeal to human interest and in part because they are among the brightest of luminous organisms. Weak lights are not only difficult to measure but, when dispersed to form spectra, give bands so faint that their limits are very difficult to see and more so to photograph. Very few organisms produce light visible to the fully light-adapted eye. Although their light may seem quite bright to the dark-adapted eye, the dark-adapted eye is a poor judge of the quality, *i.e.*, the color of a light. This is because of the Purkinje phenomenon, a change in the region of maximum sensibility of the retina with change in intensity of the light. For an equal energy spectrum, to the normal, completely light-adapted eye, yellow-green light of wave-length, $\lambda = .565\mu$, appears the brightest, but when the light is made fainter the maximum shifts first to the green and then to the blue. The dark-adapted eye can see green or blue better than yellow and for this reason weak lights will appear more green or blue than stronger ones of the same energy distribution. Also two weak lights of the same spectral composition may appear different in color if they differ much in intensity. This is illustrated in Fig. 6.

The shift in sensibility of the eye occurs in illuminations of between 0.5 and 50 metre-candles and represents a change from central cone vision (high intensities) to peripheral rod vision (low intensities). The *fovea centralis* lacks rods and this part of the eye becomes practically color blind at very low intensities of light. Below 0.5 and above 50-metre candles visibility varies but little

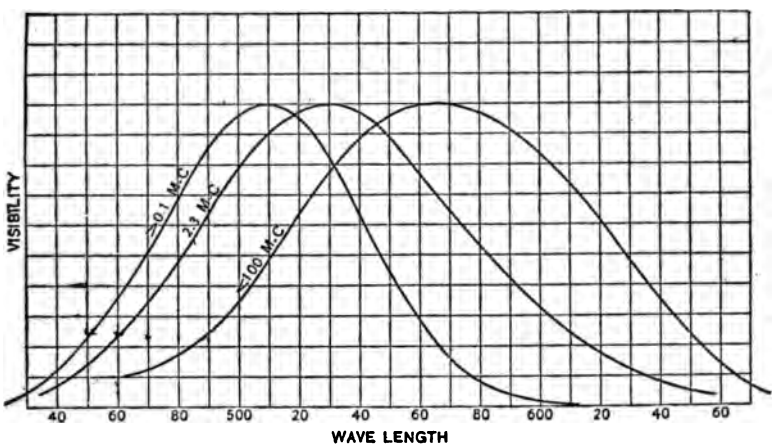


FIG. 6.—Visibility curves for three illuminations showing the shift in region of maximum visibility, or Purkinje phenomenon (*after Nutting*).

with change in intensity. It is clearly necessary then to distinguish between the physical objective phenomenon of light and the physiological subjective sensation of light.

It is a fact that different luminous animals produce light of quite different colors as judged by our eye. A range of spectral tints has been described which extends from red to violet but "yellowish," "greenish" and "bluish" tints are commonest. Indeed one or two animals possess several luminous organs emitting lights of different colors. This is true in a South American firefly, *Phen-*

godes, whose lights are red and greenish yellow, and in the deep sea squid, *Thaumatolampas diadema,* which produces lights of three colors, two shades of blue and red. The red light in the case of the squid appears to be due to a red color screen formed by the chromatophores, but in *Phengodes* no screen is present.

As we have seen, difference in color of the light does not necessarily indicate difference in spectral composition because of the Purkinje effect. However, examination of the spectrum of various luminous forms has very clearly indicated that the different colors are really due to light rays of different wave-length and are not the result of any subjective phenomena. To facilitate comparison, spectral lines and colors are given in Table 4. The first adequate observations on the spectra of luminous animals were made by Pasteur (1864), who studied *Pyrophorus* and found a continuous spectrum unbroken by light or dark bands. Lankester (1868) discovered a similar continuous spectrum in *Chætopterus insignis* and placed its limits from line 5 to 10 on Sorby's Scale (about $\lambda = 0.55\mu$

TABLE 4

Wave-lengths of Fraünhofer Lines and Prominent Lines in Line Spectra

FRAÜNHOFER LINES

Line	Color	Wave-lengths ($\mu\mu = \frac{\mu}{1000}$)	Source
A	Red	759.4 (band)	Oxygen in atmosphere.
a	Red	718.5 (band)	Water vapor atmosphere.
B	Red	686.7	Oxygen vapor atmosphere.
C	Red	656.3	Hydrogen in sun.
D_1 D_2	Yellow	589.6, 589.0	Sodium in sun.
E	Green	527.0	Calcium in sun.
b_1 b_2 b_4	Green	518.4, 517.3, 516.8	Magnesium in sun.
F	Blue	486.1	Hydrogen in sun.
G	Violet	430.8	Calcium in sun.
H K	Violet	396.9, 393.4	Calcium in sun.

BUNSEN FLAME LINES

Source	Color	Wave-lengths ($\mu\mu = \frac{\mu}{1000}$)
Potassium	Red	769.9, 766.5 (double).
Lithium	Red	670.8
Sodium	Yellow	589.6, 589.0 (double)
Thallium	Green	535.1
Magnesium	Green	518.4
Strontium	Blue	460.7

PLÜCKER TUBE LINES

Source	Color	Wave-lengths ($\mu\mu = \frac{\mu}{1000}$)
Mercury	Yellow	579.0, 576.9
	Green	546.1
	Blue	491.6, 435.8
	Violet	407.8, 404.7
Hydrogen	Red	656.3
	Blue	486.1, 434.1
Helium	Red	728.2, 706.5, 667.8
	Yellow	587.6
	Green	504.8, 501.6, 492.2
	Blue	471.3, 447.2
	Violet	438.8, 402.6, 388.8

to $\lambda = 0.44\mu$). Young (1870) first recorded the limits of the firefly spectrum as a little above *C* ($\lambda = .6563\mu$) to *F* ($\lambda = .4861\mu$). Since then a number of luminous forms have been examined and all are found to give short continuous spectra (not crossed by light or dark bands or lines) lying in different color regions. Thus, Conroy (1882) examined the glowworm (*Lampyris noctiluca*) light and observed a band extending from $\lambda = 0.518\mu$ to $\lambda = 0.656\mu$. Dubois (1886) states that the spectrum of *Pyrophorus noctilucus,* the West Indian "Cucullo," extends from slightly further than the Fraunhofer *B* line to the *F* line, while Langley and Very (1890), working on the same form, placed the limits at $\lambda = 0.468\mu$ to

$\lambda = 0.640\mu$. It consists, then, of a broad band chiefly in the green and yellow. But, "would the light not extend farther were it bright enough to be seen? . . . if the light of the insect were as bright as that of the sun would it not extend equally far on either side of the spectrum?" "It is impossible to increase the intrinsic brilliancy by any optical device, but if it be impossible to make the light of the insect as bright as that of the sun, it is on the other hand quite possible to make the light of the sun no brighter than that of the insect . . ." Langley and Very investigated this question, forming a solar spectrum from sunlight of the same intensity as that of *Pyrophorus* and a *Pyrophorus* spectrum together in the same field of the spectroscope. The latter was very much shorter than the solar spectrum, showing that its length was not due to weakness of the red and blue rays but to their absence. Later Ives and Coblentz (1910) photographed the spectrum of a firefly (*Photinus pyralis*), together with that of a carbon glow lamp, on plates sensitive to all wave-lengths of visible rays under conditions which would have recorded all visible radiations given off. They found the spectrum to extend only from $\lambda = 0.51\mu$ to $\lambda = 0.67\mu$ (Fig. 7). Another species of firefly (*Photuris pennsylvanica*) was found by Coblentz (1912) to give a spectrum extending from $\lambda = 0.51\mu$ to $\lambda = 0.59\mu$ (Fig. 8). The *Photinus* light extends much further into the red and it is easy to distinguish between *Photinus* and *Photuris* in nature, merely by the reddish tint of the light of the former. These photographic records show conclusively that the color of the light of luminous animals is not a subjective phenomenon due to the Purkinje effect and the low intensity of the light, but is real, an actual difference in spec-

tral composition of the light emitted. Neither is it due, at least in the fireflies examined, to the existence of color screens which absorb certain rays, allowing only those of a definite color to pass. The spectra of forms thus far investigated are reproduced in Fig. 9 and recorded in Table 5. It will be noted that they vary considerably in position but are all of the same type. The spectrum of *Cypridina hilgendorfii* is the longest thus far investigated ($\lambda = .610\mu$ to $\lambda = .415\mu$), extending well into the blue, and the light of this form is very blue in appearance.

As first shown by Dubois (1886) for *Pyrophorus,* and confirmed by myself for *Cypridina,* the light is not polarized in any way. I may add that the *Cypridina* light like any other light may be polarized by passing through a Nicol prism.

Several writers [Dubois (1914 book)], Fischer (1888), Molisch (1904 book) have noticed that the light of luminous bacteria changes in color if grown on different culture media. Light which is "silver white" on dead fish becomes "greenish" on salt-peptone-gelatin media and more yellow on salt-poor media. Peron (1804) and Panceri (1872) describe the light of *Pyrosoma* as yellow to greenish after death of the animal and reddish on stimulation; then fading out through orange, yellow, greenish and azure blue. Polimanti (1911) describes the normal light of *Pyrosoma* as greenish, and states that as the animals die, or if they are kept at temperatures above the optimum, the light becomes more red. McDermott (1911, *b*) noticed that the light of fireflies placed in liquid air became decidedly reddish just before going out and on rewarming the first light to appear was reddish followed by the proper shade at higher temperatures. I have frequently ob-

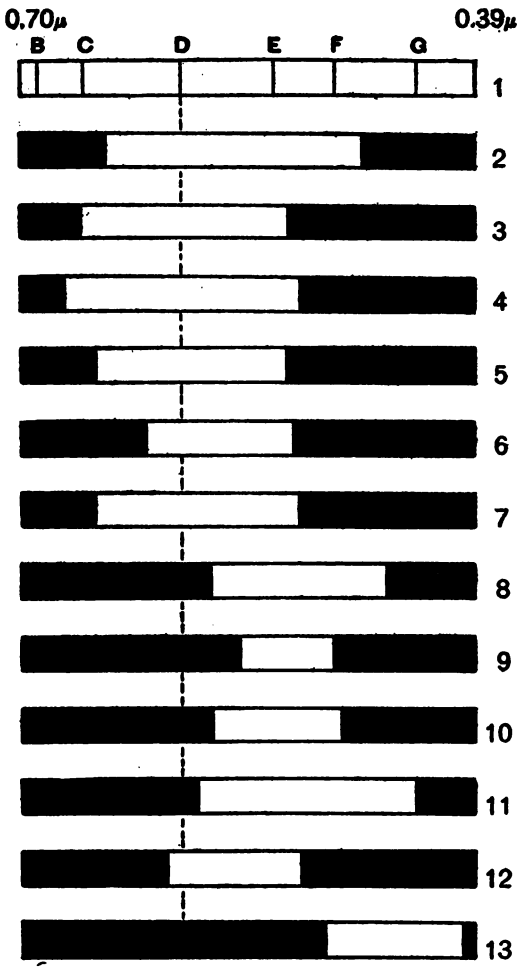


FIG. 9.—Spectra of various luminous animals (*after McDermott*). 1. Portion of the visible solar (grating) spectrum showing Fraunhofer lines. 2. *Pyrophorus noctilucus* (Langley and Very.) 3. *Lampyris noctiluca* (Conroy). 4. *Photinus pyralis* (Ives and Coblentz). 5. *Photinus consanguineus* (Coblentz). 6. *Photuris pennsylvanica* (Coblentz). 7. *Phengodes laticollis* (McDermott). 8. *Bacterium phosphoreum, B. phosphorescens or Bacillus photogenus* (Molish). 9. *Photobacterium indicum* (Barnard). 10. *Mycelium X* (Molish). 11. *Luminous bacteria* (Förster). 12. *Agaricus sp.?* (Ludwig). 13. Fluorescent spectrum of luciferesceine of *Photinus pyralis* (Coblentz). Only the extreme ends of the bands are shown and no attempt is made to indicate the relative density of different portions of the spectra.

TABLE 5.—*Limits of Spectra of Various Luminous Organisms*

Light	Spectrum (μ)	Emission maximum	Observer	Method and remarks
Cypridina hilgendorfii	0.610 −0.415	.....	Harvey, 1919	Eye observation, Zeiss comparison spectroscope.
Chætopterus insignis	0.55 −0.44 (approximately)	.....	Lancaster, 1868	Eye observation.
Pyrophorus noctilucus	0.72 −0.486	.....	Dubois, 1886	Eye observation.
Pyrophorus noctilucus (thoracic light)	.640 − .468	0.57	Langley and Very, 1890	Eye observation and comparison with solar spectrum of equal intensity.
Pyrophorus noctilucus (abdominal light)	.663 − .463	.....	.....	.....
Photinus pyralis	.67 − .51	.....	Ives and Coblents, 1909	Photographic comparison with carbon glow lamp of equal intensity.
Photuris pennsylvanica	.59 − .51	.552	Coblents, 1912	Photographic comparison with carbon glow lamp of equal intensity.
Photinus consanguineus	.65 − .52	.578	Coblents, 1912	Photographic comparison with carbon glow lamp of equal intensity.
Phengodes laticollis	.65 − .52	.....	McDermott, 1911 e	Eye observation.
Lampyris (glow worm)	.656 − .518	.....	Conroy, 1910	Eye observation.
Photinus	.670 − .487	.....	Young, 1870	Eye observation direct vision spectroscope.
Bacteria	G to F extending toward D for long exposure	.....	Barnard, 1902	Photographic.
Bacteria	Somewhat beyond G to D	.....	Fisher, 1888	Eye observation.
Bacteria	.58 − .43	.....	Forster, 1887	Eye observation Zeiss. Abbe microspectral ocular.
Bacteria	>.500 to .350	Bright band at .4	Forsyth, 1910	Photographic, quarts spectroscope.
Agaricus melleus	0.56 −0.48 (approximately)	.....	Ludwig, 1884	Eye observation, Sorby Brown microspectroscope.
Xylaria hypoxylon	.54 − .46 (approximately)	.....	Ludwig, 1884	Eye observation, Sorby Brown microspectroscope.
Micrococcus Pflugeri	b into the violet	.....	Ludwig, 1884	Eye observation, Sorby Brown microspectroscope.
Mycelium X	.570 − .480	.....	Molish, 1904, book	Eye observation, Zeiss comparison spectroscope.
Bacterium phosphoreum	.570 − .450	.....	Molish, 1904, book	Eye observation, Zeiss comparison spectroscope.
Bacterium phosphorescens	.570 − .450	.....	Molish, 1904, book	Eye observation, Zeiss comparison spectroscope.
Bacillus photogenes	.570 − .450	.....	Molish, 1904, book	Eye observation, Zeiss comparison spectroscope.
Pseudomonas lucifera	.570 − .450	.....	Molish, 1904, book	Eye observation, Zeiss comparison spectroscope.

served a more reddish color from luminous tissues of the firefly upon the addition of coagulants such as alcohol, and have noted that the light of *Cypridina* becomes weaker and more yellow at both low (0°) and high (50°) temperatures. The meaning of these color changes will be discussed in Chapter VII.

The efficiency of any light may be defined in several different ways: (1) By the percentage of visible wave-lengths in the total amount of radiation emitted, *i.e.*, visible radiation divided by total (heat, visible, actinic) radiation; (2) by considering, in addition to visible radiation ÷ total radiation, the sensibility of the eye to different wave-lengths, visible radiation × visual sensibility ÷ total radiation. Visible radiation × visual sensibility is spoken of as luminosity; (3) by the amount of light (expressed in candles) produced in relation to a given expenditure of energy or in relation to the cost of the energy expended. Thus, of the radiation emitted from an incandescent electric lamp only a small per cent. is light, the rest being heat and actinic rays. It is therefore very far from being 100 per cent. efficient. If there were no infra-red or ultra-violet in the radiation from an incandescent lamp its efficiency would be 100 per cent. if we disregarded visual sensibility. But if we take into account the fact that the eye is most sensitive to yellow green, a source of light, even though emitting only visible radiation, would not be 100 per cent. efficient unless its maximum of emission corresponded also with the maximum of visual sensibility. We shall return to this question in a later paragraph. Looking at the question from the standpoint of energy consumption, the carbon incandescent lamp gives one mean spherical candle for 4.83 watts (watt $= 10^7$ ergs

per sec.), while the tungsten lamp gives one mean spherical candle for 1.6 watts, about one-third the energy, and the latter is consequently more efficient.

As we know practically nothing of the energy transformations occurring during the process of light production in organisms, all statements regarding the efficiency of their light are based on relations between the visible radiation and total radiation. This involves a measurement of rays in the infra-red region (heat rays) and ultra-violet region (actinic rays) as well as the light rays proper, and any other radiant energy produced. While all spectroscopic investigations show that the spectrum of luminous animals never extends to the limits of the visible spectrum in either the red or violet, it is possible that bands occur in the infra-red or ultra-violet, and special methods must be employed to detect these. Radiations of all kinds, if converted into heat on striking the blackened surface of a thermopile, bolometer, or radiometer can be measured by changes in temperature and the relative amounts of energy represented be compared in a common unit, the calorie. By proper screening, all rays except the visible light rays can be cut off from the measuring instrument and the amounts of energy represented in light and in total radiation thus be determined.

Dubois (1886) first studied this problem in *Pyrophorus* by the use of a thermopile and galvanometer and found a small amount of radiation from the luminous region in excess of that from a non-luminous region. It amounted to a galvanometer deflection of 0.95° and was increased 0.3° during the flash of the insect on electrical stimulation. This increase of 0.3° is possibly due to heat produced on muscular contraction. In any case the amount of heat

radiated in comparison with that of the candle is very small indeed. A more careful study has been made by Langley and Very (1890) with the bolometer. They point out first of all that the total radiation from the most powerful luminous organ (the abdominal one) of *Pyrophorus* which affected their bolometer slightly, would, in the same time (10 seconds), be sufficient to raise the temperature of an ordinary mercurial thermometer having a bulb 1 cm. in diameter by rather less than 2.3×10^{-6}° C. We may thus gain some idea of the magnitude of the measurements to be made. The radiation from *Pyrophorus* which affected their bolometer was shown to be due merely to the "body heat" * of the insect, and it is largely cut off by a plate of glass which is opaque to all wave-lengths of 3μ or more. These waves are given off by bodies at temperatures below 50° C. and belong "to quite another spectral region to that in which the invisible heat associated with light mainly appears." Langley and Very then compared the radiation from a non-luminous bunsen flame and the *Pyrophorus* light, interposing a plate of glass in each case to cut off the waves longer than 3μ, and found several hundred times more radiation in the case of the bunsen burner but, nevertheless, perceptible radiation from *Pyrophorus*. The former consisted of radiant heat shorter than $\lambda = 3\mu$ and extending up to the visible light rays ($\lambda = 0.7\mu$, since the bunsen flame emitted no light). The very slight effect of the *Pyrophorus* radiation must be due to wave-lengths between $\lambda = 3\mu$ and $\lambda = 0.468\mu$, the limit of the *Pyrophorus* spectrum in the blue. Langley and Very assumed it to be due entirely to the band of

* Langley and Very evidently supposed that the body temperature of the firefly, like the mammal or bird, is higher than its surroundings.

visible light, $\lambda = 0.640\mu$ to $\lambda = 0.468\mu$, and assumed that no invisible heat rays were produced. All of the energy of *Pyrophorus* light would therefore lie in the visible region and its efficiency (light rays ÷ heat + light + actinic rays) would be 100 per cent. Later, Langley (1902) reinvestigated the radiation of *Pyrophorus* and could detect no heating whatever with the bolometer. "A portion of the flame of a standard sperm candle, equal in area to the bright part of the insects, gave under the same circumstances, a bolometric effect of such magnitude that had the heat of the insect been 1/80,000 as great as that from the candle, it would certainly have been recognized." Coblentz (1912) also, using a vacuum thermopile of Pt and Bi, was unable to detect any infra-red radiation from *Photinus pyralis,* but found that the temperature of this firefly is slightly lower than the air. These temperature measurements will be discussed in a later chapter.

The assumption of Langley and Very that the small amount of *Pyrophorus* radiation passing glass is all light has been called into question by Ives (1910), who points out that Langley and Very failed to use a screen which would cut off either the visible rays or the invisible rays between 3μ and 0.7μ. They really left the question open as to whether the effect of *Pyrophorus* light on their bolometer was due to the visible band of rays or to this plus another band in the infra-red. "The firefly's actual efficiency as a light source is dependent to a large degree on the radiation being confined to the visible region. If there should be found infra-red of quantity comparable to the visible, the firefly, while still a very efficient source would not be, as usually supposed, the example of an ideally efficient light produced by nature."

Ives investigated the question further by the phosphor-photographic method. "In brief it consists of this: Phosphorescence, which is excited in various substances by exposure to short waves (blue, violet or ultra-violet), is destroyed by exposure to longer waves (orange, red, infra-red). Thus, a surface of Balmain's paint or of Sidot blende, excited to phosphorescence and then exposed in a spectrograph, will have areas of reduced brightness wherever long-wave energy has fallen upon it. If this surface is then laid on a photographic plate for a short period, a permanent record is obtained on the plate after development." Preliminary tests showed that the method was applicable in the case of weak light such as the firefly spectrum and also if the light is intermittent like the firefly. With Sidot blend (ZnS) the extinguishing action extends from $\lambda = 0.6\mu$ to $\lambda = 1.5\mu$. A sheet of deep ruby glass, which cut off all the visible rays of the firefly but allowed infra-red to pass, was placed between the firefly light and a surface of phosphorescent Sidot blend which was exposed to the firefly flashes for three and a half hours. No extinction of phosphorescence occurred, while without the ruby glass, extinction, due to the orange rays of the *visible* firefly light was noticeable in 20 minutes. There is thus no infra-red of an intensity at all comparable to the visible as far as $\lambda = 1.5\mu$, the lower limit of the phosphor-photographic method. Coblentz (1912) had examined the transparency of the dry chitinous integument of various fireflies (Fig. 10) in the infra-red and reports it to be fairly transparent down to $\lambda = 2.8\mu$, opaque between $\lambda = 2.8\mu$ and $\lambda = 3.8\mu$, transparent again to $\lambda = 6\mu$, and opaque beyond that. The infra-red could, then, if it were emitted, largely pass through the integument which

is similar in absorption properties to complex carbohydrates. Transparency of the integument to the ultra-violet was not studied.

Although photographs of the spectrum of firefly (*Photinus*) light show that it extends only to the beginning of the blue, Forsyth (1910) reports ultra-violet radiation in luminous bacteria. He exposed a plate for 48 hours to

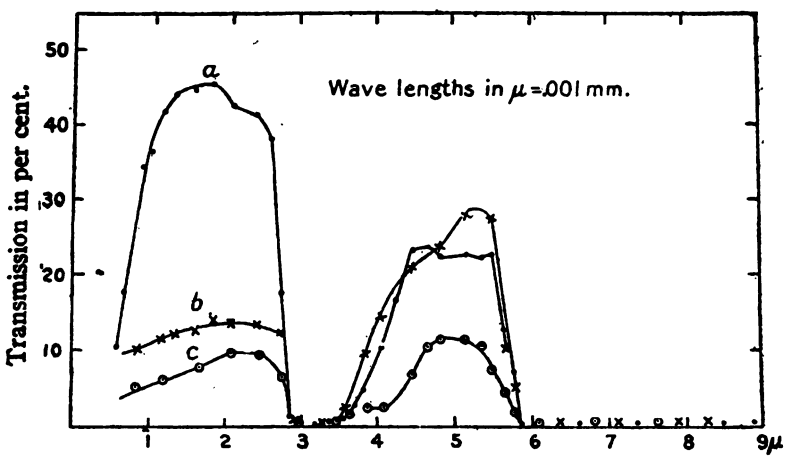


FIG. 10.—Transmissivity of the integument of fireflies to infra-red radiation (*after Coblentz.*)

the spectrum of bacterial light dispersed by a quartz prism and got a continuous band from $\lambda = 0.50\mu$ (the lower limit of sensitivity of the plate) to $\lambda = 0.35\mu$. However, McDermott (1911 *d*) was unable to observe fluorescence of p-amino-ortho-sulpho-benzoic acid, which responds to the ultra-violet light. Molisch (1904, book) photographed bacterial and fungus light through glass and through a piece of quartz and found no difference in density on the plate. As the exposure was brief, to avoid saturation, and as the ultra-violet, which passes quartz but not glass, has a much

greater action on the plate than visible light, we must conclude that ultra-violet is absent. Ives (1910) investigated the spectrum of *Photinus pyralis,* using a quartz spectroscope, and found no evidence of ultra-violet radiation, at least as far as $\lambda = 0.216\mu$.

It will thus be seen that the radiation from the firefly has been very carefully studied and that no waves are given off from $\lambda = 1.5\mu$ to $\lambda = 0.216\mu$ with the exception of the short band ($\lambda = 0.67\mu$ to $\lambda = 0.51\mu$) in the visible, and it is highly probable that no radiation is given off with wave-lengths longer than $\lambda = 1.5\mu$ The firefly light remains, then, 100 per cent. efficient, differing from all our artificial sources of light, the best of which does not approach this value. As Langley and Very express it in the title to their paper, it is "the cheapest form of light," not cheapest in the sense of that we can reproduce it commercially at less cost than other lights, but cheaper in the sense that it is the most economical in the energy radiated. This energy is all light and no heat. "Cold light" has actually been developed by the firefly and concerning which "we know of nothing to prevent our successfully imitating."

I have already pointed out that we may also consider the efficiency of a light in relation to the sensibility of our own eye. That is, we take into account not only the energy distribution in the spectrum of the light but also the fact that different wave-lengths of an equal energy spectrum affect our eye very differently. As the normal light-adapted eye is most sensitive to yellow green of $\lambda = 0.565\mu$, monochromatic light of this wave-length will appear much brighter than monochromatic light of any other wave-length with the same energy. Monochromatic

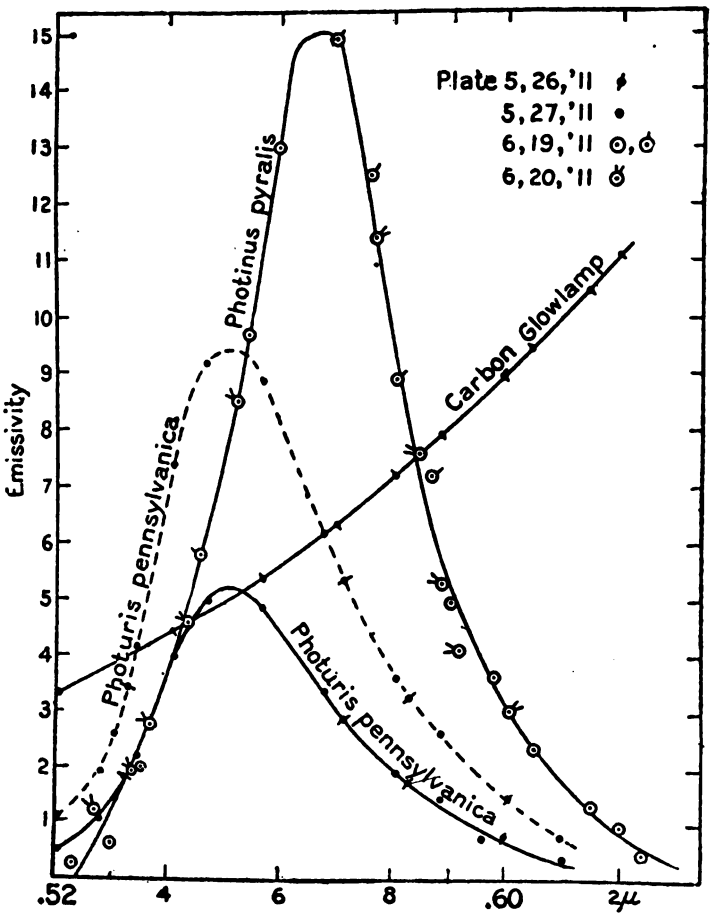


FIG. 11.—Spectral energy curves of various fireflies and the carbon glow lamp (*after Coblentz*).

light of $\lambda = 0.565\mu$ will then be the theoretically most efficient possible, when we consider the energy radiated in relation to the sensitivity of our eye. This is the usual method of determining the luminous efficiency of artificial

lights and is obtained from a knowledge of the radiated energy and the visual sensibility. Reduced luminous efficiency = light (*radiated energy* × *visual sensibility*) or luminosity ÷ total radiated energy.

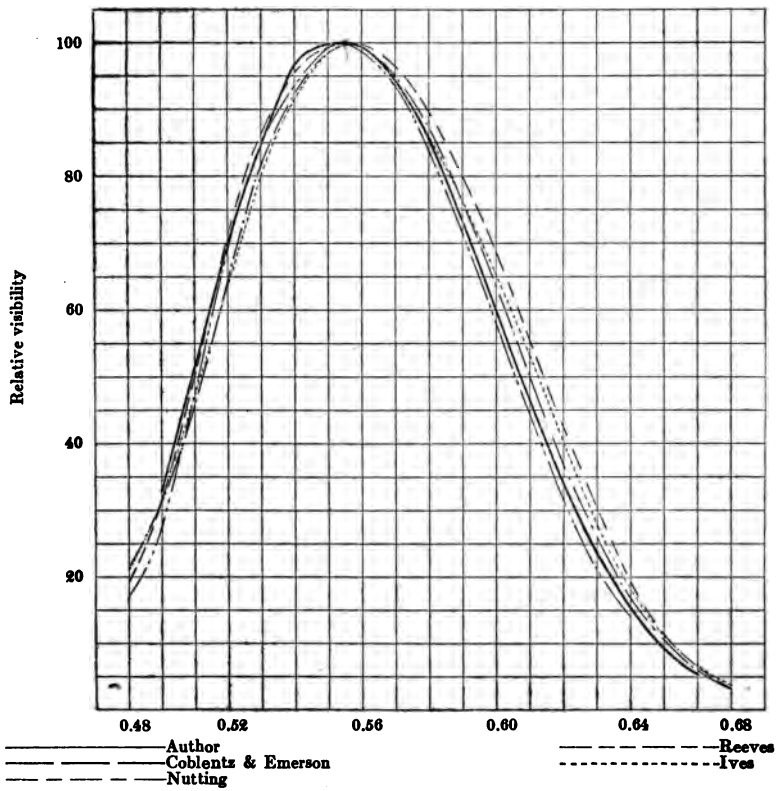


FIG. 12.—Visibility curves of various investigators obtained by different methods (*after Hyde, Forsythe and Cady*).

The spectral energy curve for the firefly has been worked out by Ives and Coblentz (1910), using a photographic method in which the intensities of different wave-lengths of the firefly (*Photinus pyralis*) light is com-

pared with that of a carbon glow lamp by measuring the amount of photochemical change produced on panchromatic photographic plates. Fig. 11 gives the energy curves of various fireflies and the carbon glow lamp in the same spectral region. The visual sensibility curve used by Ives and Coblentz is that of Nutting (1908, 1911), based on Konig's data. It is reproduced in Fig. 6. The latest visibility curve is that of Hyde, Forsyth and Cady (1918),

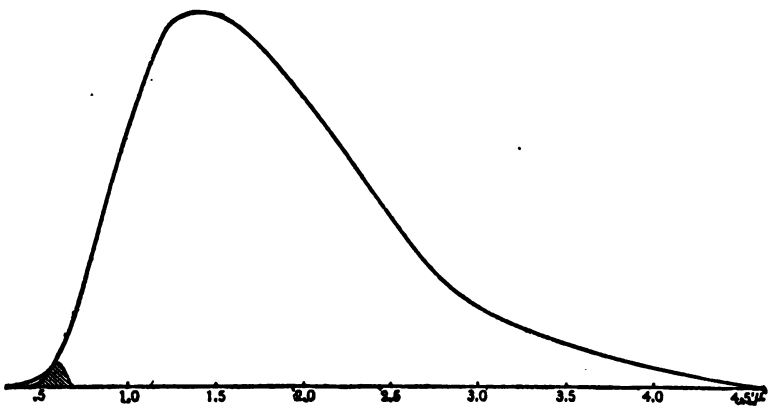


FIG. 13 —Luminous efficiency of the 4-watt carbon glow lamp, shaded area ÷ total area (*after Ives and Coblentz*).

reproduced in Fig. 12. It is based on observations of twenty-nine individuals. As individuals vary considerably in their sensibility to different wave-lengths, the visibility curve represents an average, but it is the only standard we have with which to evaluate the energy we call light. Color-blind individuals would have a visibility curve very different from normal individuals. Composite curves showing the luminous efficiency of the 4-watt carbon glow lamp and the firefly, both in relation to visibility, are given in Figs. 13 and 14, respectively. In these figures

the luminous efficiency is the shaded area ÷ total area, 0.43 per cent. for the carbon glow lamp and 99.5 per cent. for the firefly, "these numbers representing the relative amounts of light (measured on a photometer) for equal amounts of radiated energy—a striking illustration of the

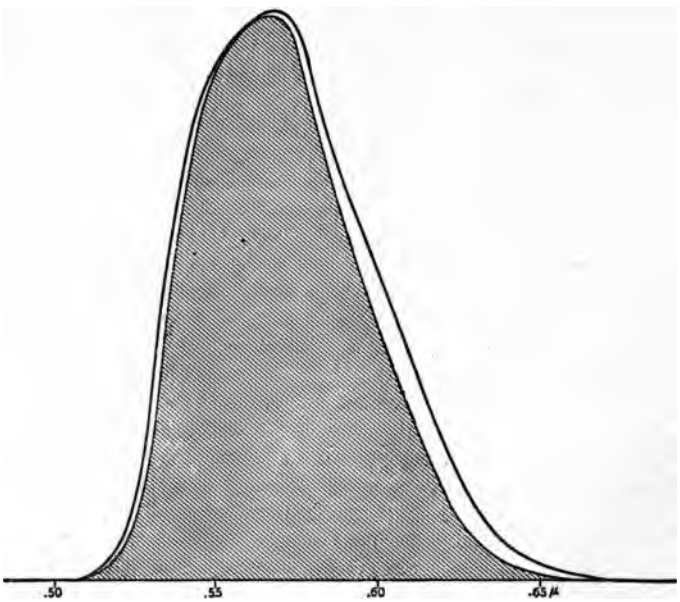


FIG. 14.—Luminous efficiency of the firefly, shaded area ÷ total area (*after Ives and Coblentz*).

wastefulness of artificial methods of light production. From the specific consumption of the tungsten lamp (1.6 watts per spherical candle) and the mercury arc (.55 watts per spherical candle) we obtained by comparison with the carbon filament that their luminous efficiencies are 1.3 and 3.8 per cent. The most efficient artificial illuminant therefore has about 4 per cent. of the luminous efficiency of the

firefly." This is calculated to be .02 watts per candle. More recent determinations (Coblentz, 1912), using a new sensibility curve of Nutting's (1911) for a partially light-adapted eye, give the reduced luminous efficiency as 87

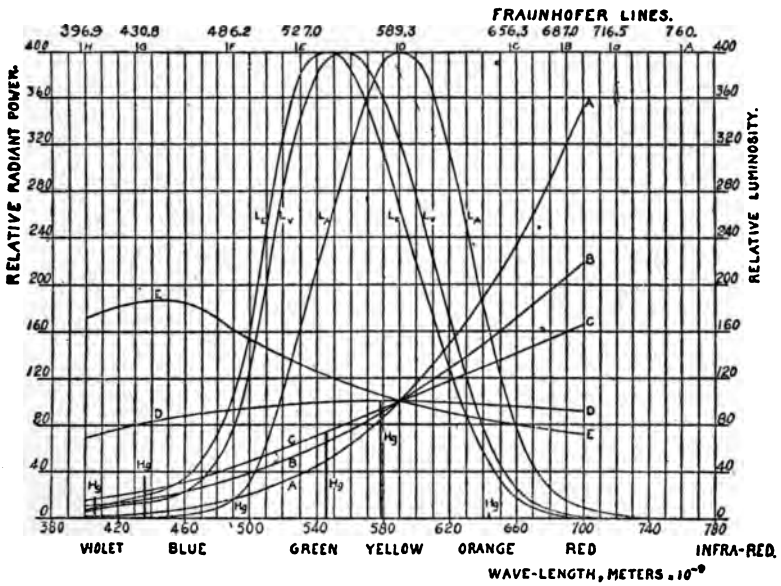


FIG. 15.—Spectral energy, luminosity and visibility curves (*after Gibson and McNicholas*)
A. Spectral energy curve of Hefner lamp.
B. Spectral energy curve of acetylene flame.
C. Spectral energy curve of tungsten (gas-filled) glow lamp.
D. Spectral energy curve of black body at 5000° absolute (sunlight).
E. Spectral energy curve of blue sky.
Hg. Spectral energy curve of Heræus quartz mercury lamp.
Lv Visibility curve for human eye.
La. Luminosity of Hefner lamp.
Le. Luminosity of blue sky.

per cent. for *Photinus pyralis,* 80 per cent. for *Photinus consanguineus* and 92 per cent. for *Photuris pennsylvanica.*

The luminous efficiencies of various forms of artificial illuminants have been calculated by Ives (1915) and are given together with that of the firefly in Table 6. Fig. 15

gives spectral energy curves for various illuminants reduced to 100 at $\lambda = .590\mu$, luminosity curves for the Hefner lamp and blue sky, and a visibility curve worked out by Coblentz and Emerson (1917) from observations on 130 individuals.

TABLE 6

Luminous Efficiencies of Various Illuminants

Illuminant and commercial description	Commercial rating	Lumens per watt	Efficiency (visible radiation × visual sensibility ÷ total radiation)
Carbon incandescent lamp oval anchored (treated) filament	4 watts per mean horiz. c.	2.6	0.0042
Tungsten incandescent lamp, vacuum type	1.25 watts per mean horiz. c.	8.0	.013
Mazda, type c	600 C. P. 20 amp., 0.5 w. p. c. Series type C.	19.6	.032
Carbon arc (open)	9.6 amp. clear globe	11.8	.019
Open arc, yellow flame, inclined trim	10 amp. D. C.	44.7	.072
Quartz mercury arc	174–197 volt, 4.2 amp.	42.0	.068
Glass mercury arc	40–70 volt, 3.5 amp.	23.0	.037
Nernst lamp		4.8	.0077
Acetylene	1 L per hr. consumption	.67	.0011
Petroleum lamp		.26	.0004
Open flame gas burner	Bray 6 high pressure	.22	.00036
Incandescent gas lamp, low pressure	.350 lumens per B. T. U. per hr.	1.2	.0019
Incandescent gas lamp, high pressure	.578 lumens per B. T. U. per hr.	2.0	.0032
Firefly		629.0	.96

The firefly light by the above method of calculating efficiency is not 100 per cent. efficient because its maximum ($\lambda = 0.567\mu$) does not correspond with the maximum sensibility of the eye ($\lambda = 0.565\mu$), but taking into consideration also other effects of color, the firefly light would be a still more inefficient and trying one for artificial illumination, as all objects would appear a nearly uniform

green hue. Indeed the distortion would be even greater than with the mercury arc, whose objectionable green hue is so well known. "We may say, therefore, that the firefly has carried the striving for efficiency too far to be acceptable to human use; it has produced the most efficient light known, as far as amount of light for expenditure of energy is concerned, but has produced it at the (inevitable) expense of range of color. The most efficient light for human use, taking into account both color and energy-light relationships, would be a light similar to the firefly light containing no radiation beyond the visible spectrum, but differing from it by being white." (Ives, 1910.) Although the spectral energy curve for *Cypridina* light has not been worked out, it will be noted that the *Cypridina* spectrum is much longer than that of the firefly, more nearly approaching the spectrum of an incandescent solid giving white light. It approaches, but does not attain the ideal.

Although Muraoka (1896) and Singh and Maulik (1911) have described radiations coming from fireflies which would pass opaque objects and affect a photographic plate, and Dubois reports the same from bacteria, the existence of such radiation has been denied by Suchsland (1898), Schurig (1901) and Molisch (1904 book). The experiments of Molisch on luminous bacteria are of greatest interest, for they are very carefully controlled and show without a doubt that black paper or Zn, Al, or Cu sheet will allow no rays from these organisms to pass that will affect a photographic plate, even after several days' exposure. The *visible* light of luminous bacteria will affect the plate after one second exposure. Moreover, Molisch has pointed out the errors of those who claim to

have found penetrating radiation in luminous forms. It seems that certain kinds of cardboard, especially yellow varieties, or wood, will give off vapors that affect the photographic plate. The action is especially marked with damp cardboard at a temperature of 25°–35° C., and Dubois and Muraoka must have used such cardboard to cover their plates. A piece of old dry section of beech or oak trunk, placed on a photographic plate for 15 hours in a totally dark place, will register a beautiful picture of the annual rings of growth, medullary rays, junction of bark and wood, etc. Russell (1897) had previously found that many bodies, both metals and substances of organic origin (gums, wood, paper, etc.), placed in contact with photographic plates, would affect them, and concluded that vapors and not rays were the active agents. As a dry piece of wood has a very definite smell, there is something given off which can affect our nose and there is no reason why it should not change, by purely chemical action, the photographic plate. This action of wood on the plate is prevented by interposing a sheet of glass. Frankland (1898) has described similar vapors coming from colonies of *Bacillus proteus vulgaris* and *B. coli communis* which affect a photographic plate laid directly over the colonies in an open petri dish. There is no effect if the glass cover of the petri dish is between plate and bacteria. There is, then, no specific emission of X-rays or similar penetrating radiation from luminous tissues which will affect the photographic plate through opaque screens.

A similar conclusion is reached if we attack the problem in another way. X-rays and radium rays (Becquerel rays) cause fluorescence of ZnS, barium platinocyanide,

willemite (Zn_2SiO_4), and calcium tungstate. Coblentz (1912) showed that the firefly will cause no fluorescence of a barium platinocyanide screen and I have been unable to detect fluorescence of zinc sulphide, barium platinocyanide, zinc silicate (willemite) or calcium tungstate shielded from *Cypridina* light by black paper, although the light of this organism is quite bright enough to cause phosphorescence of zinc sulphide without the black paper. The samples of the above four substances all showed fluorescence in presence of radium rays, but only the ZnS phosphoresces after exposure to light rays, although the willemite was phosphorescent after exposure to the ultra-violet.

While photometry at low intensities is a difficult procedure at best, if the light varies in intensity or is a flash, accurate measurements become well-nigh impossible. The figures given for intensity of animal luminescence must, therefore, be accepted with a realization of the difficulties of measurement. By candle is meant the international candle, unless otherwise specified, equal to 1.11 Hefner candles (H. K.) 0.1 pentane lamp and 0.104 carcel units. It is a measure of intensity.

Amount of light, or light flux, measured in lumens, is that emitted in a unit solid angle (area/r^2) by a point source of one candle-power. One candle-power emits 4π lumens. The latest figure for the mechanical equivalent of light at $\lambda = .566$ is .0015 watt (Hyde, Forsyth and Cady, 1919), *i.e.*, 1 lumen = .0015 watt. One watt is 10^7 ergs (one joule) per second.

The illumination (of a surface) is that given by one candle at one metre, the candle metre (C.M.) or lux. The

surface then receives one lumen per square metre. A metre kerze (M.K.) is the illumination given by one Hefner candle at one metre distance.

The brightness of a surface is measured in lamberts or millilamberts. A lambert is "the brightness of a perfectly diffusing surface radiating or reflecting one lumen per square cm." A millilambert is 1/1000 lambert. For further definitions the reader is referred to the reports of the committee on nomenclature of the Illuminating Engineering Society.

Dubois (1886) states that one of the prothoracic organs of *Pyrophorus noctilucus* has a light intensity of 1/150 Phœnix candle of eight to the pound (probably about equivalent to 1/150 candle) and that 37 or 38 beetles (each using all three light organs) would produce light equivalent to one Phœnix candle. Langley (1890) found that to the eye the prothoracic organ of *Pyrophorus noctilucus* gave one-eighth as much light as an equal area of a candle and the actual candle-power of the insect was 1/1600 candle. It may be remarked in passing how widely divergent these observations are.

For the flash of the firefly (*Photinus pyralis*) Coblentz (1912) found variation from 1/50 to 1/400 candle, the predominating values being around 1/400 candle. A continuous steady glow is sometimes obtained from this insect and it proved to be of the order of 1/50,000 candle.

Steady sources of light can be more easily measured and we have two records of the light intensity from luminous organisms with continuous light. One of these is a fish, *Photoblepharon palpebratus,* with a large luminous organ under the eye, of flattened oval shape, 11 × 5 mm., which glows continuously without change of intensity.

The organ can be darkened by a screen similar to an eyelid which pulls up over it. Steche (1909) reports the intensity to be .0024 M.K.*

Luminous bacteria probably glow with less intensity than any other organism. The light from a single organism cannot be seen but that from a colony is visible to the dark-adapted eye. Even so we must remember that the eye is an exceedingly delicate instrument which can detect very small energy changes. The "minimum radiation visually perceptible" has been calculated by Reeves (1917) to be in the neighborhood of 18×10^{-10} ergs per second and the light from a small colony of luminous bacteria represents little more radiation than this.

Lode (1904, 1908), by a modified grease spot photometer method, ascertained that the light of his brightest bacterial colony of *Vibrio rumple* had an intensity of 7.85×10^{-10} H.K. per sq. mm. or 0.785 H.K. per 1000 sq. metres (=0.562 German-normal candles per 1000 sq. metres). In round numbers this is about one German-normal candle per 2000 sq. metres, or two to three times this area for the light from an ordinary stearin candle. Lode calculated that the dome of St. Peter's at Rome, if covered with bacteria, would give little more light than a common stearin candle. An ordinary room of 50 sq. metres wall and ceiling area would give out only 0.039 German-normal candle. It does not seem likely that luminous bacteria will ever come into vogue for illuminating purposes. Friedberger and Doepner (1907) by a photographic method, not entirely free from error, found that one square millimetre of lighting surface of a bouillon culture

* The metre-kerze is a unit of illumination, not of intensity, and is incorrectly used by Steche.

of photobacteria gave 6.8×10^{-9} German-normal candles, about ten times Lode's value. Even at this rate commercial lighting by luminous bacteria does not appear a promising field for investors.

To sum up, we may say that light from animal sources is in no way different from light of ordinary sources, except in intensity and spectral extent. It is all visible light, containing no infra-red or ultra-violet radiation or rays which are capable of penetrating opaque objects. It is not polarized as produced, but may be polarized by passing through a Nichol prism. Like ordinary light, animal light will also cause fluorescence and phosphorescence of substances, affect a photographic plate, cause marked heliotropism of plant seedlings (Nadson, 1903) and stimulate the formation of chlorophyll (Issatschenko, 1903, 1907). Because of the weakness of bacterial light, etiolated seedlings do not become green to the eye (Molisch, 1912 book), but a small amount of chlorophyll is formed which can be recognized by the spectroscope because of its absorption bands.

CHAPTER IV

STRUCTURE OF LUMINOUS ORGANS

THE production of light is the converse of the detection of light. In the first case chemical energy is converted into radiant energy; in the second case radiant energy is converted into chemical energy. The lantern of the firefly is an organ of *chemi-photic* change; the eye is an organ of *photo-chemical* change. While it is theoretically probable that all reactions which proceed in one direction under the influence of light, will proceed in the opposite direction with the evolution of light, the formation of luciferin from oxyluciferin (described in Chapter VI) is the only one definitely known. Perhaps we may place in this category also the instances of photoluminescence, but the chemical reaction involved cannot be pointed out.

We know of no animal whose eyes, the organs, *par excellence,* of photochemical change, give off light in the dark. All cases of luminous eyes have been conclusively shown to be purely reflection phenomena. The eyes of a cat only glow if some stray light is present which may enter and be reflected out again. Photochemical reactions and chemiluminescent reactions do have this in common, however, that they are largely but not exclusively oxidations. Whether all photochemical changes in the eyes in animals require oxygen or not, is unknown, but all animal light-producing reactions, without exception, are oxidations, and light is only produced if oxygen is present. Some material is oxidized.

In general, we may divide luminous organisms into two great classes according as the oxidizable material is burned within the cell where it is formed or is secreted to the exterior and is burned outside—intracellular and extracellular luminescence. Many animals with intracellular luminescence have quite complicated luminous organs. It is an interesting fact that a great similarity may be observed between the evolution of the complex organs of vision and of these complicated organs. In the simplest unicellular forms certain structures within the cell serve as the photochemical detectors of light, while in luminous protozoa, similarly, granules scattered throughout the cell are oxidized with light production. In the higher forms the eye contains groups of photosensitive cells connected with afferent nerves, lenses, and accessory structures for properly adjusting the light, while luminous organs contain groups of photogenic cells in connection with efferent nerves, lenses, and accessory structures for properly directing the light. It is interesting to note that in the two groups where the eye has attained its highest development, the cephalopods and vertebrates, here also the luminous organ is found in greatest complexity and perfection. In intermediate stages of evolution the eye and luminous organ so closely approach each other in structure that it is still a mooted question whether certain organs found in worms and crustacea are intended for receiving or producing light.

We may also divide luminous forms into two groups according as the oxidation of luminous material goes on continuously, independently of any stimulation of the organism; or is intermittent, oxidation and luminescence occurring only as a result of stimulation, using the word

"stimulation" in the same sense in which it is used in connection with nerve or muscle tissue. Bacteria, fungi, and a few fish produce light continuously and independently of stimulation. Its intensity varies only over long periods of time and is dependent on the nature of the nutrient medium or general physiological condition of the organism. All other forms give off no light until they are stimulated. Stimulation may of course come from the inside (nerves) or outside. Only under unfavorable conditions, such as will eventually lead to the destruction of the luminous cells, do these forms give off a continuous light. This has often been spoken of as the "death glow," and is to be compared with *rigor* in muscle tissue.

Some of the fish which produce a continuous light possess a movable screen similar to an eyelid which can be drawn across the organ, thus shutting off the light, so that the animal appears to belong to the group which flashes on stimulation. This is true of *Photoblepharon,* while *Anomalops* can rotate the light organ itself downward, so as to bring the lighting surface against the body wall and thus cut off the light (Steche, 1909). Other fish (*Monocentris*) are unable to "turn off" their light.

Animals which flash spontaneously on stimulation through nerves from within, possess a very varied rhythm. The different species of fireflies can be distinguished by the character of their flashing (McDermott, 1910–17; Mast, 1912). Fig. 16 shows the method of flashing of some common eastern North America species. The glow-worm light lasts for many seconds and then dies out. This interval of darkness persists for some minutes and is then followed by another period of glowing. Some fireflies have a light which may be described as partially intermittent.

It lasts for hours, but may become more dim or be intensified on stimulation.

Some forms only produce light at certain seasons of

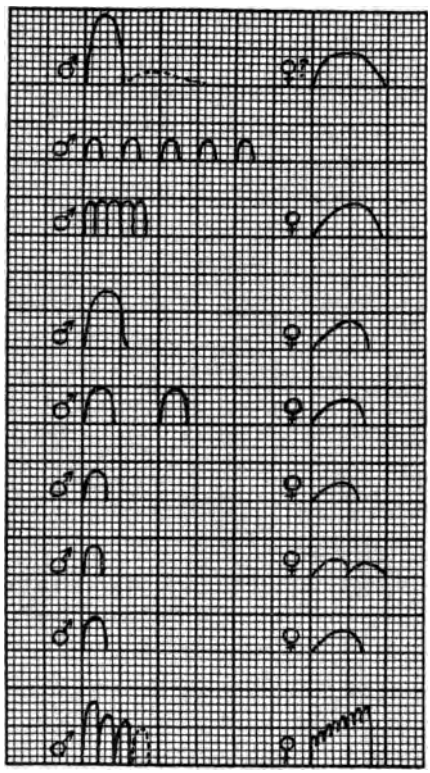


FIG. 16.—Chart showing relative intensities and durations of flashes of American fireflies (*after McDermott*). One cm. vertically = approximately 0.02 candle power; one cm. horisontally = approximately one second. The flash of the males (♂) is at the left; that of females (♀) at right of chart.

the year. According to Giesbrecht (1895) this is true of the copepods, which only light in summer and autumn, and according to Greene (1899) in the toad-fish; *Porich-*

thys, which can only be stimulated to luminesce during the spawning season in spring and early summer.

Some animals possess a periodicity of luminescence. They only luminesce at night and fail to respond to stimulation or are difficult to stimulate during the day. Bright light has an inhibiting effect. Perhaps correlated with this is the fact that most luminous forms are strongly negatively heliotropic. Fireflies lie hidden in the day, to appear about dusk and the ostracod crustacean, *Cypridina,* is difficult to obtain on moonlight nights.

The Ctenophores were the first forms in which the inhibiting effect of light was noticed. This was described by Allman (1862) and has been confirmed by a number of observers, especially Peters (1905). Massart found that *Noctiluca* was difficult to stimulate during the day and *Ceratium,* according to both Zacharias (1905) and Moore (1908), only luminesces at night, or if kept in darkness, for some little time. Crozier * finds a persistent day-night rhythm of light production when *Ptychodera,* a balanoglossid, is maintained for eight days in continued darkness. The animal is difficult to stimulate during the period which corresponds to day and luminesces brilliantly and at the slightest touch during the period which corresponds to night.

On the other hand, a great many forms are able to luminesce quite independently of previous illumination. According to Crozier * *Chætopterus* luminescence is not affected by an exposure to 3000 metre-candles for six hours.

In the case of animals with extracellular luminescence we may speak of luminous secretions and true luminous

* Private communication.

glands. A large number of forms possess luminous glands or gland cells, including some of the *medusæ,* the hydroids (probably), the pennatulids (?), the molluscs (*Pholas* and *Phyllirhoë* (probably), some cephalopods (*Heteroteuthis* and *Sepietta*), most annelids, ostracods, copepods, some schizopods (*Gnathophausia*) and decapod (*Heterocarpus* and *Aristeus*) crustaceans, all myriapods, and the balanoglossids. The remaining organisms burn their material within the cell. These include the bacteria, fungi, protozoa, some medusæ (?), ctenophores (probably), most cephalopods, a few annelids (*Tomopterus* (?)), ophiuroids(?), some schizopod (*Nyctiphanes, Euphasia, Nematocelis, Stylochiron*) and decapod (*Sergestes*) crustacea, all(?) insects, *Pyrosoma,* and fishes (*selachians* and *teleosts*). It is among this latter type that the most complicated luminous organs have been developed. While a description of all the types of luminous organs and luminous structures cannot be attempted here (excellent descriptions have been given by Dahlgren and Mangold) it is necessary to understand the structural conditions in a few of the forms whose physiology has attracted most attention.

Luminous bacteria are so small that the light from a single individual cannot be seen. It is almost impossible to make out structural differences within the cell and we cannot definitely state in just what special region, if any, the luminescence is produced. We do know that the light is intracellular and that filtration of the bacteria from their culture medium gives a dark sterile filtrate absolutely free from any luminous secretion.

Among protozoa, in certain forms at least, it is easy to observe that luminescence is connected with globules or

granules which were considered by the earlier observers to be oil droplets. Thus, in *Noctiluca* (Figs. 17 and 18), when the animal is violently stimulated or in the presence of reagents which slowly kill it, the whole interior appears a mass of starry points of light which can be traced to minute granules along the strands of protoplasm (Quatrefages, 1850).

Turning to the multicellular forms, we find the simplest development of luminosity in those animals which possess gland cells producing a luminous secretion. These cells may be scattered over the surface of the animal as in *Chætopterus* (Fig. 19) or *Cavernularia,* or restricted to certain areas [*Pholas,* (Fig. 19),] or more definitely localized to form an isolated group of gland cells as in *Cypridina.* True multicellular glands also occur. In every case, however, we find that the luminosity of these uni- or multicellular glands is connected with the presence of granules. They are often spoken of as *luciferine granules,* although it is not certain whether they are made up of luciferin or luciferase (see Chapter IV) or both. They are most similar to the zymogen granules found so abundantly in gland cells and thought to be the precursors of various enzymes. According to Dahlgren (1915), the luciferine granules stain blue-black by iron hæmatoxylon after fixation at the boiling point, and photogenic cells can be detected by this method of selective staining. Dubois (1914, book), who regards them as examples of *bioprotein,* comparable to the chondriosomes and handed on from one generation to another, gives them the name of *vacuolides* or *macrozymases.* In some forms he has described their transformation into crystals and believed at one time that animal light was a crystalloluminescence. His figures of the

crystal transformation are not very convincing. Pierantoni (1915) has considered the granules to be *symbiotic* luminous bacteria, but this is certainly not the case.

The light of *Chætopterus* comes from a material mixed with a mucous secretion formed over almost the whole body surfaces of the animal. A section of the epithelium shows large mucous-producing cells and smaller granule-containing light cells (Fig. 20). These appear to be under nervous control, as a strong stimulation in one part of the body causes luminescence which spreads over the whole surface of the worm. The animal becomes fatigued rather readily, however. In the pennatulids, such as *Cavernularia,* we have also the formation of a luminous secretion over the whole surface of the body and the individual animals in this colonial form are also connected with nerves. A stimulation in any local region, as Panceri (1872) first showed (Fig. 21), will cause a wave of luminosity to spread from this point until it extends over the whole surface of the colony. In *Pennatula* the rate of this luminous wave is about 5 cm. per second.

Pholas dactylus possesses similar light cells to those of *Chætopterus,* but they are restricted to narrow bands on the siphon and mantle and a pair of triangular spots near the retractor muscles. Nerves pass to the luminous regions.

In many luminous animals the light secretion formed over the surface of the body is small in amount and adheres to the animal because it is embedded in the mucous skin secretions. In those forms which possess a true localized light gland the luminous secretion when expelled into the sea water (if the animal be a marine form) may persist as a luminous streak for some time and exhibit

diffusion and convection movements. The most beautiful examples of luminous secretions are found among the ostracod crustacea.

In *Cypridina hilgendorfii* the luminous gland is situated on the upper lip near the mouth. It is made up of elongate (some 0.7 mm. in length), spindle-shaped cells, each one of which opens by a separate pore with a kind of valve. The openings are arranged on five protuberances. Muscle fibres pass between the gland cells in such a way that by contracting the secretion can be forced out. In the sea water the secretion luminesces brilliantly and the Japanese call these forms *umi hotaru,* or marine fireflies. Fig. 22 is a diagram showing the structure. Watanabe (1897), who first studied this form, and also Yatsu (1917) have described two kinds of granule-containing cells, one with large yellow globules, 4–10μ in diameter (Fig. 23), the other with small colorless granules 0.5, in diameter. I have observed in the living form these two types and also large colorless globules of the same size as the yellow globules. All dissolve when extruded into the sea water. Dahlgren * has described from sections four types of cells containing (1) large globules, (2) small granules, (3) a fat-like material, (4) a mucous material. Just what the significance and nature of these types of substance is cannot be stated at present. At least one, probably two, are concerned in light production The others may possibly form digestive fluids which act on the food of the animal.

Turning now to the animals possessing light cells with intra-cellular luminescence we find in general that such light cells are localized to form definite light organs and

* Private communication soon to be published.

that these may be single, as in the common fireflies, paired, as the prothoracic light organs of *Pyrophorus,* or scattered over the surface of the body, as in so many shrimps,

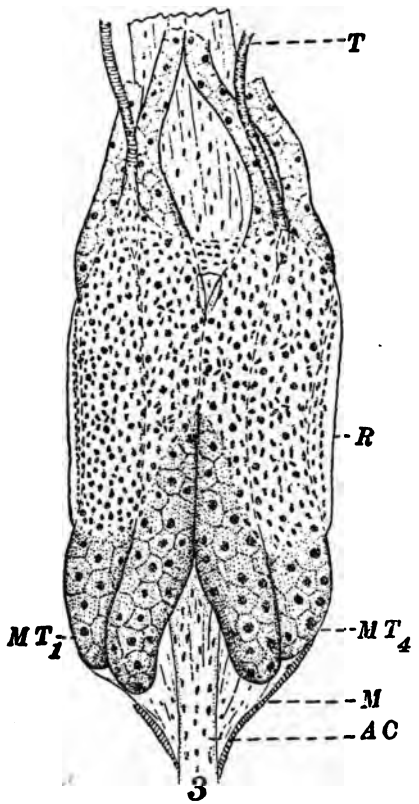


FIG. 24.—Distal portion of malpighian tubule of *Bolitophila*, showing modification to form photogenic organ (*after Wheeler and Williams*). MT_1, MT_4, malpighian tubules forming photogenic organ; *R*, reflector; *M*, muscle; *T*, trachea.

cephalopods and fishes, when they are often called photophores. The light cells proper are often associated with reflectors, lenses, opaque screens and color screens.

The insects possess the simplest types of intracellular light organs, a mass of photogenic cells, which, in the common firefly (*a lampyrid beetle*) of Eastern North America, has probably been developed from the fat body, while in the New Zealand glowworm, the larva of a tipulid fly (*Bolitophila luminosa*), part of the Malpighian tubule cells have acquired photogenic power (Wheeler and Williams, 1915). This is illustrated in Fig. 24.

The photogenic organ of the firefly is made up of two kinds of cells, a dorsal mass of small cells several layers deep, the reflector layer, and a ventral mass of large cells with indistinct boundaries, the photogenic layer (Fig. 25). The photogenic cells contain a mass of granules, spherical in the male and short rods in the female. The photogenic cells are divided into groups by large tracheal trunks which pass into the light organ and branch to form tracheoles connected with tracheal end cells. The exact distribution varies in different species, but in all the arrangement is such as to give a very abundant oxygen supply. Each group of photogenic cells is surrounded by a clear ectoplasm containing no granules. The tracheoles pass through this and either end openly within the photogenic cells or anastomose with tracheoles from neighboring tracheæ. Nerves, but no blood-vessels—which are absent in insects—enter the organ. It is difficult to determine if the nerves supply the tracheal end cells or the photogenic cells.

The dorsal reflecting layer is made up of cells containing numerous minute crystals of some purin base, either xanthin or urates, or both. They have a white milky appearance and while they are certainly not good reflectors in the optical sense, they do act as a white back-

ground, scatter incident light, and partially prevent its penetration to the internal organs of the firefly. Although a few crystals similar to those of the reflector layer are found in the photogenic cells and in other cells of the body, it is known that the photogenic cells are not transformed into the reflector cells. The two layers are distinct and permanent from an early stage in development.

Curiously enough, the light organ of the larva of the firefly (glowworm) is quite distinct from that of the adult. Like so many other structures in insects, the adult organ is developed anew from potential photogenic cells during the pupal period. Even the egg of the firefly is luminous and glows with a steady light, and during the pupal period light may sometimes be seen coming from the thoracic region.

In the firefly there is no true lens, the light merely shining through the cuticle which is transparent over the light organ, whereas over the rest of the body it is dark and pigmented. In the deep sea shrimp, *Acanthephyra debelis,* with light organs scattered over the surface of the body, the cuticle covering the light organ forms a concavo-convex lens, behind which are the photogenic cells (Kemp, 1910). As may be seen from Fig. 26, the lens is made up of three layers which suggests that it may be corrected for chromatic aberration—a veritable "achromatic triplet." In an allied form, *Sergestes* (Fig. 27), the lens is of two layers and double convex. Optical studies of these lanterns have been made by Trojan (1907). The course of the light rays is shown in Fig. 28. The lens of these organs is also bluish in color which suggests that they may serve also as color filters. Behind the photogenic cells is a mass of connective tissues through

which enters the nerve, for the light of these organs is under the control of the animal and may be flashed "at will."

All gradations in complexity of light organs may be found from the condition in the shrimp just described to that found among the squid and fish. Figs. 29 and 30 are

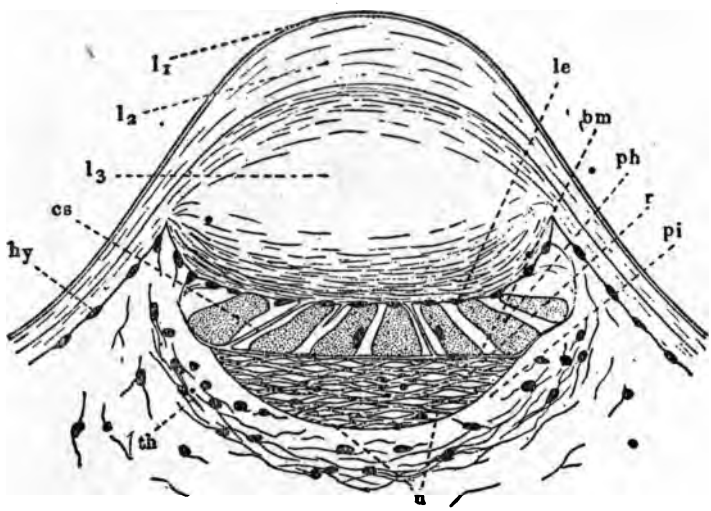


FIG. 27.—Sectional view of photogenic organ of *Sergestes prehensilis* (*after Terao*). *bm*, basement membrane; *cs*, connective strands of photogenic layer; *hy*, hypodermis; l_1, l_2, l_3, layers of lens; *le*, lens epithelium; *n*, nerve; *ph*, photogenic cells; *pi*, pigment layer; *r*, reflector; *th*, theca.

sections of two of the more complicated types found in squid. The explanation given to the various structures is that of Chun (1903) to whom we are indebted for a careful histological investigation of these forms. It will be noted that in addition to photogenic and lens tissues there are various types of reflector cells and a line of pigment about the whole inner surface of the organ to effectively screen the animal's tissues from the light. In one form (Fig.

30) chromatophores are found about the region where the light is emitted and these no doubt serve as color filters.

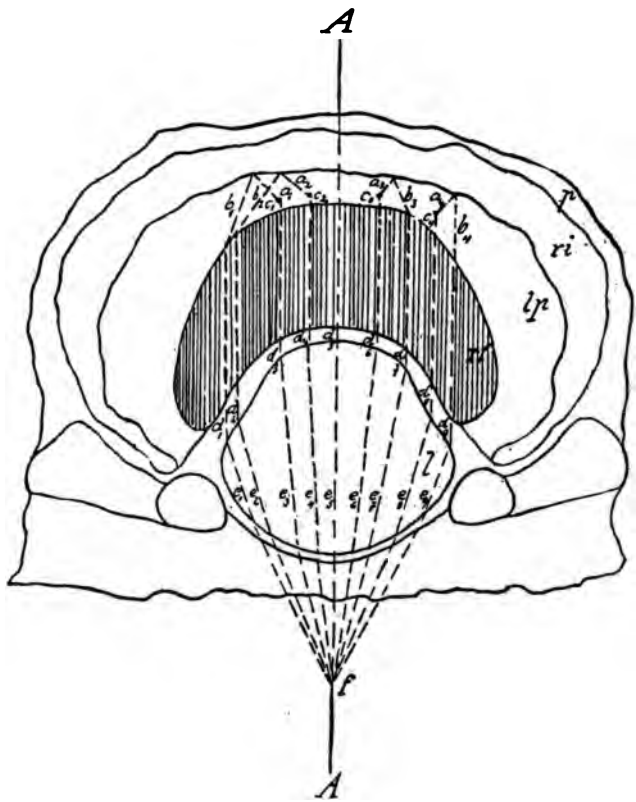


FIG. 28.—Diagram of photogenic organ of *Nyctiphanes Conchii*, to show pathways of light rays arising in the light cell layer (*after Trojan*). *p*, pigment; *ri*, inner reflector; *lp*, light cells; *rf*. refractor; *f*, focus; *l*, lens; *A-A*, axis; a_1–a_4, b_1–b_4, light rays reflected from *ri*; c_1–c_4, light rays passing directly outward; d_1–d_3 and e_1–e_3, light rays which have passed refractor and lens respectively.

There are also an abundant blood supply and nerves passing to the organ. Figs. 30 and 31 are sections through light organs of fishes.

We thus see that light organs may be very simple and

also very complicated. The latter must have evolved from the former, although it is not always possible to point out the intermediate stages. It is not within the scope of this book to discuss bioluminescence in its evolutionary aspects. It may be worth while, however, to point out

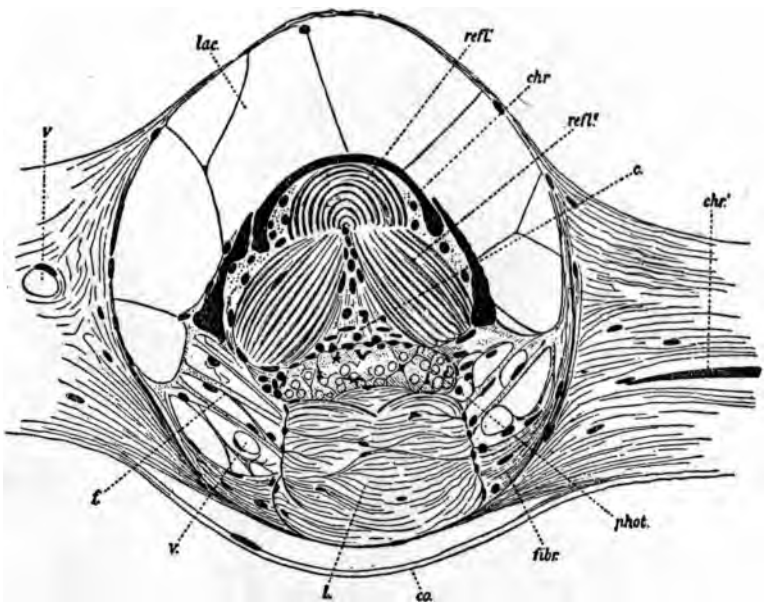


FIG. 29.—Sectional view of photogenic organ of a squid, *Abraliopsis* (*after Chun.*) *refl*¹, *refl*², reflectors; *lac.*, lacunar spaces; *chr.*, pigment screen of chromatophores; *chr.*¹ chromatophore; *phot.*, photogenic cells; *l*, lens; *co.*, cuticle; *v*, blood vessel; *fibr.*, connective tissue.

briefly what is known concerning the use of the light to the animal. There are four possibilities.

(1) The light may be of no use whatever, purely fortuitous, an accompaniment of some necessary or even unnecessary chemical reaction.

This appears to be the case in the luminous bacteria and fungi and perhaps the great majority of forms which

FIG. 32.—Sectional view of photogenic organ of a fish, *Argyrophelecus affinis* (*after Brauer*). *p*, pigmented screen; *dr.*, photogenic cells; *r*, *r*¹, reflector ?; *l*, lens ?; *s*, sclera; *g*, connective tissue.

fungi but *Noctiluca* and other animals are not known in a non-luminous condition, although we can see no definite value to the organism of this power of luminescence.

In the case of sea pens, however, we might suppose that the light acts as an attraction to small organisms on which the sea pen feeds, although these creatures only luminesce when stimulated in some way, which rather detracts from the above suggestion.

(2) The light may act as a warning to scare away predacious animals which would otherwise feed on the luminous organism. Perhaps this is the case in the sea pens, although these forms possess nematocysts which should serve as adequate protection. The marine worm, *Chætopterus,* is brightly luminous and lives its whole life in an opaque parchment tube. If this tube were torn open by a predacious form we might conceive that the attacking animal would be alarmed by the light and refrain from destroying the worm. The *Chætopterus,* however, could not rebuild another tube and its light would only protect it in the night time. These cases will suffice to indicate the difficulties and perplexities of the problem. Perhaps we may add one more guess and suppose that the light of certain fishes is actually for blinding or distracting their enemies or blinding the forms on which they feed. Until this use of luminous organs has actually been observed, we can give little credence to it.

(3) The light may serve as a means of recognition or a sex signal to bring the sexes together for mating. It would seem from the work of Mast and of McDermott that this is the case in the common fireflies and it may be the case in the toad-fish, *Poricthys,* which is only luminous in the spawning season and in the worm, *Odontosyllis,* of

Bermuda, which is brilliantly luminous while swarming when the eggs and sperm are shed. It is non-luminous at other times (Galloway and Welch, 1911.)

(4) Finally, it is possible that animals with complex luminous organs, such as squid, fish and shrimp, actually use these as lanterns. It is significant that most of them are deep sea forms, living in a region of perpetual darkness, and it is perfectly logical to suppose that they make use of their light organs for illuminating purposes.

The whole problem of the use and purpose of luminous organs is an exceedingly complex and difficult one. We have, perhaps, said enough to indicate this and may add that in most cases, so far as opinion is based on actual evidence and observation, that of the layman is of as great value as that of the scientist.

CHAPTER V

THE CHEMISTRY OF LIGHT PRODUCTION, PART I

Two experiments, both performed very early in the history of Bioluminescence, are of great importance in understanding the nature of animal light. Boyle (1667), as already mentioned, proved the necessity of air for the luminescence of wood and fish and Spallanzani (1794) showed that parts of luminous medusæ gave no light when dried but if moistened again would emit light as before. We see then, that air (oxygen), water, and some photogenic substance are necessary for the light production. Spallanzani's experiment, which has been confirmed for a great many luminous forms, shows also that animal luminescence is not a *vital* process, in the same sense that the conduction of a nerve impulse is a vital process. A nerve loses its characteristic property of conduction on drying or maceration while luminous cells still possess the power to luminesce after drying or maceration. Using the terminology of the older physiology we may say that "living protoplasm" is not necessary for light production.

The experiments of Boyle (1626–91) are of great interest, especially those in which he studied the behavior of shining wood under the receiver of his air pump. On October 29, 1667, he wrote:

"Exp. I.: Having procured a Piece of *shining Wood,* about the bigness of a groat or less, that gave a vivid Light, (for *rotten Wood*) we put it into a middle sized

Receiver, so as it was kept from touching the Cement; and the *Pump* being set a-work, we observed not, during the 5 or 6 first Exsuctions of the Air, that the splendor of the included Wood was manifestly lessened (though it was never at all increased;) but about the 7th Suck, it seemed to glow a little more dim, and afterwards answered our Expectation, by losing of its Light more and more, as the Air was still farther pumped out; till at length about the 10th Exsuction, (though by the removal of the Candles out of the Room, and by black Cloaths and Hats we made the place as dark as we could, yet) we could not perceive any light at all to proceed from the *Wood.*

"Exp. II.: Wherefore we let in the outward Air by Degrees and had the pleasure to see the seemingly extinguished Light revive so fast and perfectly, that it looked to us almost like a little Flash of Lightning, and the Splendor of the Wood seemed rather greater than at all less, than before it was put into the Receiver."

Boyle proved that light from the wood was able to pass a vacuum and later showed that "shining fish" behaved as the "shining wood," but that a piece of white hot iron would not regain its light on readmitting air to the exhausted receiver and that the iron lost its glow under the air-pump merely because it cooled off. A piece of glowing coal, however, did lose its light in the absence of air and regained it on again admitting air, provided the air had not been removed for too long. Boyle was apparently impressed with the similarity of the light giving process in glowing coal and shining wood as he draws a comparison between the two which brings out the fundamental similarity of combustion processes.

"Resemblances:

VII. The Things wherein I observed a Piece of *shining Wood* and a *burning Coal* to agree or *resemble* each other are principally these *five:*

1. Both of them are *Luminaries,* that is, give *Light,* as having it (if I may so speak) *residing in them;* and not like *Looking-glasses,* or *white Bodies,* which are conspicuous only by the *incident Beams* of the *Sun,* or some other *luminous Body,* which they *reflect.* . . .
2. Both *shining Wood* and a *burning Coal* need the Presence of the Air (and that too of such a *Density* to make them continue *shining.* . . .
3. Both *shining Wood* and a *burning Coal,* having been deprived, for a Time, of their *Light,* by the withdrawing of the contiguous *Air,* may presently recover it by letting in fresh *Air* upon them. . . .
4. Both a *quick Coal* and *shining Wood* will be easily quenched by *Water* and *many other Liquors.* . . .
5. As a *quick Coal* is not to be *extinguished* by the Coldness of the *Air,* when it is greater than ordinary; so neither is a Piece of *shining Wood* to be deprived of its *Light* by the same Quality of the *Air.* . . .

Differences:

1. The first Difference I observed betwixt a *live Coal* and a *shining Wood* is, that whereas the *Light* of the *former* is readily *extinguishable* by *Compression* (as is obvious in the Practice of suddenly

extinguishing a piece of *Coal* by treading upon it), I could not find that such a *Compression* as I could conveniently give without losing sight of its operation, would *put out,* or much injure the *Light,* even of small Fragments of *shining* Wood. . . .

2. The next *Unlikeness* to be taken notice of betwixt *rotten Wood* and a *kindled Coal* is, that the latter will, in a very few *Minutes,* be totally *extinguished* by the withdrawing of the *Air;* whereas a Piece of *shining Wood,* being eclipsed by the Absence of the *Air,* and kept so for a Time, will immediately *recover* its *Light* if the *Air* be let in upon it again within half an hour after it was first withdrawn. . . .
3. The next *Difference* to be mentioned is, that a *live Coal,* being put into a small close Glass, will not continue to *burn* for very many *Minutes;* but a Piece of *shining Wood* will continue to shine for *some* whole *Days.* . . .
4. A *fourth Difference* may be this: that whereas a *Coal,* as it *burns,* sends forth Store of *Smoke* or *Exhalations, luminous Wood* does not so.
5. A *fifth,* flowing from the former, is, that whereas a *Coal* in *shining* wastes itself at a great Rate, *shining Wood* does not. . . .
6. The last Difference I shall take notice of betwixt the bodies hitherto compared is, that a *quick Coal* is actually and vehemently hot; whereas I have not observed *shining Wood* to be so much as sensibly *lukewarm.*"

It should be clearly borne in mind that if we place luminous organisms, say bacteria or fungi, in an atmosphere devoid of oxygen and find that no light is produced, this may merely mean that certain functions of the cell are interfered with, including light production, but does not necessarily indicate that oxygen is actually used up in the photogenic process. If we find, however, that extracts of luminous cells or luminous secretions devoid of cells cease to light when the oxygen is removed and again luminesce when it is returned, we may be quite certain that the photogenic process itself requires free oxygen. As luminous extracts of fireflies, pennatulids, ostracods, *Pholas* and others give off no light when the oxygen is removed, we may safely conclude that for these luminescences, oxygen is necessary. Bacteria, fungi, and *Noctiluca,* whose light also disappears in absence of oxygen, although they are whole cells, we may by analogy also assume to require oxygen in the photogenic process.

Some of the earlier workers on fireflies and *Noctiluca* obtained light even after placing these organisms in absence of oxygen, but they did not realize how low is the amount of oxygen necessary to produce light. It is difficult to remove traces of oxygen from the water, traces which are nevertheless sufficient to cause luminescence. If the organisms are numerous, as in an emulsion of luminous bacteria, they will themselves use up all the oxygen and the liquid soon ceases to glow except at the surface in contact with air. We may gain an idea of the amount of oxygen necessary for luminescence from an experiment of Beijerinck (1902). He mixed luminous bacteria with an emulsion of clover leaves containing chloroplasts and kept the two in the dark until all the

oxygen was used up and the bacteria ceased to glow. If now a match was struck for a fraction of a second, sufficient oxygen was formed by photosynthesis to cause the bacteria to luminesce for a short time.

Exact figures on the minimal concentration of oxygen for luminescence cannot be given. The luminescent secretion of *Cypridina hilgendorfii* will still give off much light if hydrogen containing only 0.4 per cent. of oxygen is bubbled through it, *i.e.*, a partial oxygen pressure of 1/250 atmosphere (3.04 mm.Hg). However, addition of a fresh emulsion of yeast cells to a glowing *Cypridina* secretion is sufficient to rapidly extinguish the light, because the yeast is capable of utilizing the last trace of oxygen in the mixture. Light only appears when, by agitation, we cause more air to dissolve. The minimal concentration of oxygen for luminescence of *Cypridina* lies somewhere between 3.04 mm. and the amount which living yeast fails to extract from solution, a concentration approaching zero. It is probably nearer the latter figure.

As the oxygen pressure is increased from 0 to about 7 mm., the intensity of the *Cypridina* luminescence increases and at the latter figure the light is just as bright as if the solution were saturated with air (152 mm.0_2). Thus, the luminescence requires only a low pressure of oxygen and the similarity to the saturation of hæmoglobin with oxygen is obvious. Just as hæmoglobin is nearly saturated with oxygen at low pressures and becomes bright red in color, so the luminous material becomes saturated with oxygen at low pressures and glows intensely.

Boyle also made many experiments to show that air was necesary for the life of animals and the germination

of seeds and showed that repeatedly respired air was unfit for further breathing. About the same time R. Hooke discovered the true meaning of respiratory movements and by forcing a blast of air continuously through the lungs with bellows, was able to keep animals alive. He concludes "that as the bare Motion of the *Lungs,* without fresh air, contributes nothing to the life of the Animal, he being found to survive as well as when they were not moved as when they were; so it was not the Subsiding or Movelessness of the *Lungs* that was the immediate cause of death, or the stopping of the circulation of the Blood through the *Lungs,* but the Want of a sufficient Supply of fresh Air." The cause of death on collapse of the lungs could not be better stated to-day. Thus combustion, respiration and luminescence of flesh or wood were early recognized as related phenomena.

Although the "gas sylvestre" (CO_2) of burning charcoal and fermentation of wine was known to van Helmont (1577–1644) and Mayow (1646–1679) in 1674 showed that "spiritus nitroærens" (oxygen) was responsible for the life of animals and for combustion, a century elapsed before the true significance of these gases became known. In the meantime the phlogiston theory of combustion had been developed, Black (1728–1799) in 1755 had rediscovered carbon dioxide ("fixed air") in the expired air and Priestley (1733–1804) and Scheele (1742–1786) had both rediscovered oxygen ("dephlogisticated air") in 1774. About the same time Lavoisier overthrew the phlogiston doctrine and showed that in the combustion of organic substances water and CO_2 are formed.

Later it was realized that this slow combustion did not take place in the lungs, or in the blood, but in the

tissues cells themselves and respiration in the chemical sense has come to mean this universal slow combustion in the cells of the body rather than the breathing movements of the lungs themselves. In anaerobic respiration, CO_2 is given off, but no oxygen absorbed. In aerobic respiration, oxygen is absorbed and CO_2 given off. In addition we know of many substances which oxidize by taking up oxygen without giving off CO_2. We have seen that oxygen must be absorbed for luminescence of animals and we may now inquire whether CO_2 is given off and the relation between respiration and light production.

To determine if CO_2 is given off during luminescence it is necessary to work with fairly pure luminous materials, obtained from luminous organisms. It is impossible to use the living organisms themselves as the CO_2 continually respired becomes a very disturbing factor. From *Cypridina,* a small crustacean, two materials soluble in water may be prepared (*luciferin* and *luciferase*), which will give a brilliant luminescence on mixing. It is possible to determine the H-ion concentration of the two solutions separately and of the mixture of the two after the luminescence has occurred.

If CO_2 is produced during luminescence the H-ion concentration of the luminous solution should increase. Measurements made electrometrically with the hydrogen electrode have failed to demonstrate any increase in acidity. The P_H of both solutions and of a mixture of the two is 9.04. This would indicate that CO_2 is not produced. As both luminous solutions contain proteins and the luminous substances themselves are probably proteins, which have a high buffer value, a method of this kind is none too sensitive. However, we can definitely state that not enough

CO_2 is produced to be detected and that this may be due to the buffer action of the luminous substances themselves. After all, unless luminescence is connected with respiration, we should hardly expect CO_2 to be produced.

Another method of testing CO_2 production is to measure the amount of heat produced during luminescence. Substances burned during respiration give off considerable heat, one gram of glucose to CO_2 and H_2O, as much as 4000 calories. We have seen in Chapter III that no infrared radiation is produced in the light of the firefly. This does not mean, however, that no heat is produced by the reaction which produces the luminescence. A temperature change of a few thousandths or hundredths of a degree would evolve no measurable radiation. Coblentz (1912) first studied the problem of heat production in the firefly, using a thermo-couple as the measuring instrument. He came to the conclusion that the temperature of the insect was slightly lower than the temperature of the air and that the luminous segments were slightly hotter than the non-luminous segments, whereas a dead firefly is of the same temperature as its surroundings. No definite increase or decrease in temperature could be established during the flash of the firefly. However, further work on the firefly is much to be desired.

The use of a living animal for such measurements introduces a possible source of error in that any contraction of the muscles of the animal will produce heat which may add to an increase or mask a decrease of temperature during luminescence. Utilization of extracts of luminous animals containing the luciferin and luciferase mentioned above avoids the complications due to muscular contraction. By bringing the solutions of luciferin and luciferase

to the same temperature and then mixing them one can measure any increase or decrease of temperature which occurs during the luminescence which results from mixing. We can thus gain some idea of the heat of oxidation of luciferin.

As a determination of heat production is of considerable interest the method will be given in some detail. Although the experiment sounds very simple, it is actually somewhat difficult to carry out. The attainment of temperature equilibrium between two solutions is very slow when one wishes to obtain them to within 0.001° C. of the same temperature. After many attempts, the following arrangement of apparatus (Fig. 33) was found most satisfactory. About 10 c.c. luciferin solution was placed in the inner tube (*D*) of a special non-silvered thermos bottle (*A*). About 1 c.c. of luciferase solution was placed in a very thin-walled glass tube (*E*) which was immersed in the luciferin solution and connected with a small motor so that it could be slowly but constantly rotated, thus stirring the solutions. Thermo-couples (*L* and *M*) of advance (.008 in)—copper (No. 30, *B* and *S*, enamel insulated) wire were paraffined and placed in each tube and the copper wires connected through a copper double throw switch (*C*) with a Leeds and Northrup d'Arsonval wall galvanometer (No. 34637, silver strip suspension) of 35 ohms resistance and 310 megohms sensitivity. The constant temperature junctions (*N*) were placed in a large Dewar flask (*D*) filled with water at approximately the same temperature as the luciferin solution. One mm. galvanometer scale division represented 0.003° C. and the division readings could be estimated to tenths. By means of a glass rod (*F*) placed in

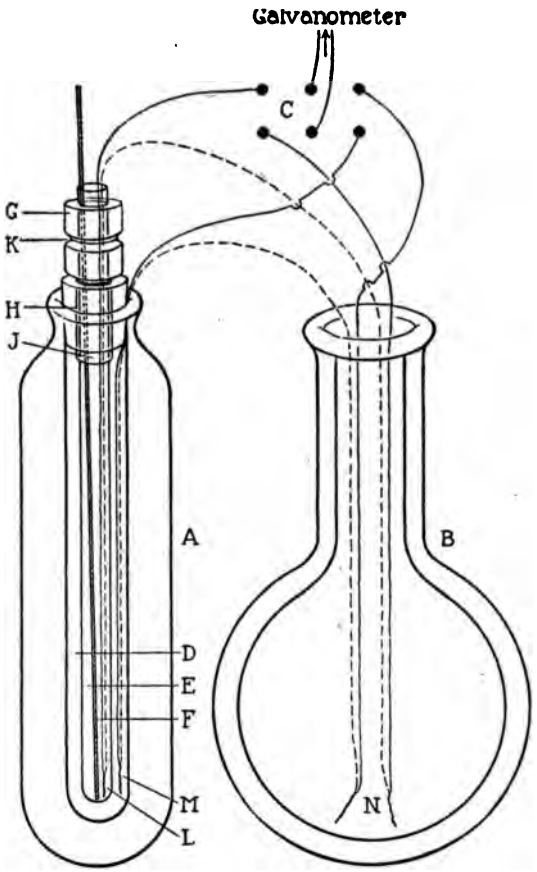


FIG. 33.—Apparatus for determining heat production during luminescence of luciferin. A, special thermos tube. B, Dewar flask for constant temperature junctions. C, double throw switch. D, tube containing luciferin solution. E, tube containing luciferase solution. F, glass rod for breaking E. G, rubber stopper with groove, K, for pulley cord. H, cork closing thermos tube. J, brass sleeve in H allowing rotation of E. L, thermojunction in luciferase solution. M, thermojunction in luciferin solution. N, constant temperature junctions.

the tube containing luciferase solution, this tube could be broken and the luciferase and luciferin solution mixed.

It was found that even after the luciferase and luciferin

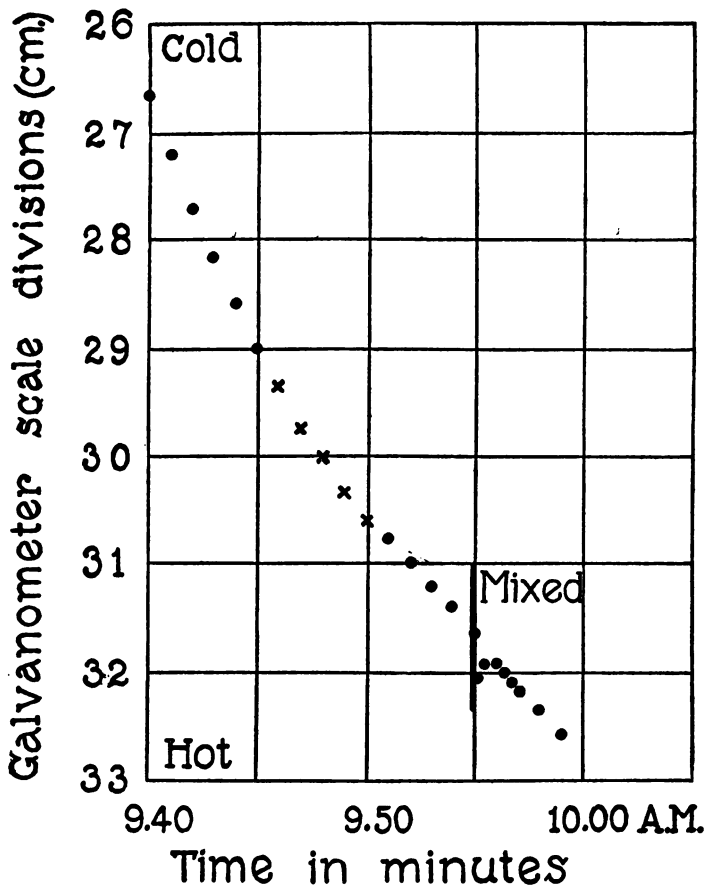


FIG. 34.—Curve showing temperature change when two tubes containing water at the same temperature are mixed. 0.1 galvanometer scale division = 0.003° C. Dots represent readings of thermocouple in tube D; crosses readings of thermocouple in tube E.

solutions came to the same temperature within the thermos bottle, this was not necessarily the same as that of the room and a slow rise or fall occurred as indicated by a slow drift of the galvanometer coil. Readings of each

thermo-couple on the galvanometer scale were therefore taken at one-minute intervals for some time before and after mixing the luciferin and luciferase solutions and plotted as curves. Control experiments were also carried out in exactly the same manner as the luciferin-luciferase experiments, but water was placed in the two tubes instead

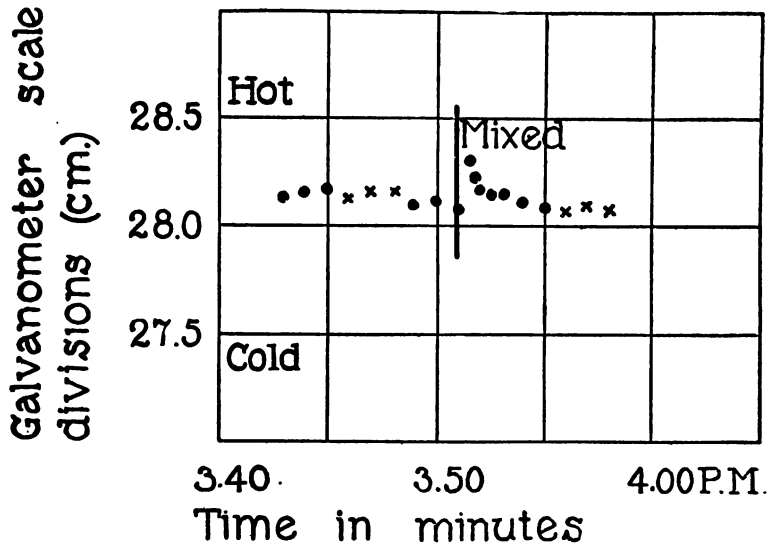


FIG. 35.—Curve showing temperature change when luciferin and luciferase solutions at the same temperature are mixed. 0.1 galvanometer scale division = 0.003° C. Dots represent readings of thermocouple in luciferin solution; crosses, readings of thermocouple in luciferase solution.

of luciferin and luciferase. Figs. 34 and 35 give typical experiments with water and with luminescent solutions, respectively.

With both control (water) and luciferin experiments there was a slight rise in temperature on mixing the liquids in the two tubes. The average rise of five control (water) experiments was .0054° C. and the average rise of five luciferin experiments was .0048° C.

The average rise in temperature is no doubt due to heat from friction in mixing of the liquids and breaking of the glass tube. The difference in the average rise of control and of luciferin experiments is so small (.0006° C.) as to have little significance. We may therefore conclude that if any temperature change occurs during the luminescent reaction it is certainly less than 0.001° C. and probably less than 0.0005° C., too small to be measured by this method.

To prepare the luciferin solution, two grams of dried *Cypridina* were dissolved in 20 c.c. hot water and 10 c.c. of this 10 per cent. solution was used in the thermos bottle in the above experiments. If we assume that 1 per cent. of the dried *Cypridina* is luciferin, 0.01 gram of luciferin on oxidation was not able to raise the temperature of the 10 c.c. (in reality 11 c.c., since 1 c.c. luciferase solution was mixed with the 10 c.c. luciferin solution) .001° C. This means that 1 gram luciferin liberates *at least less* than 10 calories during the luminescence accompanying oxidation.

Since 1 gram glucose liberates 4000 calories on complete oxidation to CO_2 and H_2O, it will be seen that the oxidation of luciferin is a very different type of reaction from the oxidation of glucose. As we shall see, it is probably similar to the oxidation of reduced hæmoglobin or the oxidation of leuco methylene-blue to methylene blue. According to Barcroft and Hill (1910), 1.85 calories are produced per gram of hæmoglobin oxidized. I have been unable to find figures for the heat exchange during oxidation of leuco-dyes, but it is no doubt also small. Since luciferin evolves no measurable amount of heat on oxidation, we have very good evidence in support of that

obtained by electrometric measurements of H-ion concentration, that no carbon dioxide is produced during luminescence of luminous animals.

In most animal cells it is perfectly clear that luminescence does not accompany respiration, since respiration is a continuous process, whereas light is only produced on stimulation. It is true that on stimulation respiration is accelerated, and we might suppose that luminescence is an accompaniment of accelerated respiratory oxidations; but this is not the case, for in luminous animals a rise in temperature of ten degrees centigrade will accelerate the respiratory oxidations 250 per cent. without necessarily causing the production of light.

In fungi and bacteria, on the other hand, which continually emit light, it is quite natural to suppose that the light is an accompaniment of respiration, just as we know the heat of these forms to be. This view was accepted by such of the earlier workers as Fabre in 1855, who found that luminous portions of a mushroom, *Agaricus olearius,* gave off more CO_2 (4.41 c.c. CO_2 per gram in 36 hours at 12° C.) than non-luminous portions (2.88 c.c. CO_2 per gram in 36 hours at 12° C.). This experiment has never been repeated and there are many reasons besides luminescence why one piece of fungus might have a more rapid respiratory rate than another piece. It is not true that rapidly respiring plant tissues, such as germinating seeds or the spadix of *Araceæ,* are luminous, although they produce considerable heat.

On the other hand, it is very easy to prove that luminescence, even in bacteria, is not connected with respiration. Thus, Beijerinck (1889 *c*) found that of several species of luminous bacteria studied by him, one, *Bac-*

terium phosphorescens, was a facultative anaërobe and would grow, *i.e.,* multiply, but not luminesce in the absence of oxygen. Some forms, ordinarily producing light, will grow, but fail to luminesce at high temperatures. Beijerinck (1915) has recently found that these individuals may, by continued cultivation at high temperatures, form non-luminous strains which fail to luminesce when again brought into lower temperatures, favorable for luminescence. These non-luminous mutants occasionally give rise to atavistic brilliantly luminous forms. Beijerinck also finds that after exposure of *Photobacter splendidum* to ultra-violet or strong sunlight, radium or mesothorium rays, luminescence continues but no growth occurs. There is thus ample evidence that growth and respiration are properties quite distinct and separable from luminescence. Indeed, respiration increases continuously up to a relatively high maximum whereas luminescence falls off rapidly above a relatively low optimum. McKenny (1902) found also that *Bacillus phosphorescens* could grow rapidly in 0.5 per cent. ether without producing light.

Luminescence has been compared in bacteria to pigment formation, as rather definite cultural conditions are necessary for realization of both chromogenic and photogenic function. Some pigment-formers, as *Bacillus pyocyaneus,* which produces a water-soluble green pigment, remain colorless under anaërobic conditions. A colorless chromogen is formed, which oxidizes to the green pigment in the air. If this colorless chromogen produced light during its oxidation as well as green pigment, we would have a case of both chromogenic and photogenic function combined in one species of bacterium. Luminescence in-

volves something more than respiration, an oxidation of a very definite and particular kind.

Since Spallanzani's observation that the luminous material of medusæ could be dried, and upon moistening would again give light, many confirmatory observations have been made on other forms. *Pyrosoma, Pholas, Phyllirrhoë,* fireflies, *Pyrophorus,* copepods, ostracods, pennatulids, fungi, and bacteria can all be dessicated and the photogenic material preserved for a greater or less time. In a dessicator filled with $CaCl_2$, dried luminous bacteria lose, after a few months, their power to give light on being moistened. On the other hand, ostracods and copepods will still luminesce after years of dessication. The luminous material in the latter case appears capable of indefinite preservation, but it is possible that the quick loss of photogenic power with dried luminous bacteria is merely an indication that they contain very little photogenic substance and that the dried ostracods would also in time lose their power to luminesce. It is certainly a fact that the amount of luminous material in a single gland cell of an ostracod is vastly greater than that in the same mass of bacterial colony.

When the dried powdered luminous material of an ostracod is sprinkled over the surface of water, it goes into solution and leaves luminous diffusion and convection trails plainly visible in the water. Many luminous marine forms give off a phosphorescent slime when they are handled, which adheres to the fingers. It is not surprising that this luminous matter should have early received a name. In 1872, Phipson called it *noctilucin* and described some of its properties. He regarded the luminous matter which can be scraped from dead fish (luminous

bacteria) and the mucous secretion of *Scolopendra electrica* or the luminous matter of the glowworm to be this material, noctilucin, which, "in moist condition, takes up oxygen and gives off CO_2 and when dry appears like mucin." Phipson says that it forms an oily layer over the seas in summer (he probably refers to masses of dinoflagellates), is liquid at ordinary temperatures and less dense than water, smells a little like caprylic acid, is insoluble in water but misceable with it, insoluble in alcohol and ether, dissolves with decomposition in mineral acids and alkalies and contains no phosphorus. We can see from this description that the word "noctilucin" does not indicate a chemical individual, but it is the earliest attempt to definitely designate the luminous substance.

The idea of a definite substance oxidizing and causing the light has been upheld by a number of investigators, and many years later Molisch called this substance the *photogen.* He contrasts the "photogen theory" with certain other views of light production, which may be spoken of as "vital theories," notably those of Pflüger (1875), who looked upon luminescence as a sign of intense respiration, and of Beijerinck (1915), who regarded the light as an accompaniment of the formation of living matter from peptone.

Fortunately biological science has advanced beyond the stage where a living process can be explained by calling it a vital process, and we must fall back upon the idea of a photogen oxidizing with light production. Indeed, it is now possible to go much further than this and describe the properties of the photogen, but we must not lose sight of the fact that it was recognized very early in the history of Bioluminescence, that water,

oxygen, and a photogenic substance were necessary for light production.

A very great advance in our knowledge of the chemistry of the problem was made by Dubois in 1885. He showed that if one dips the luminous organ of *Pyrophorus* in hot water, the light disappears and will not return again. Also if one grinds up a luminous organ the mass will glow for some time but the light soon disappears. If one brings the previously heated organ in contact with the unheated triturated organ it will again give off light. Later, Dubois showed that the same experiment could be performed with the luminous tissues of *Pholas dactylus*. A hot-water extract of the luminous tissue, and a cold-water extract of the luminous tissue, allowed to stand until the light disappears, will again produce light if mixed together. Dubois (1887 *b*) advanced the theory that in the hot-water extract there is a substance, luciferin, not destroyed by heating, which oxidizes with light production in the presence of an enzyme, luciferase, which is destroyed on heating. The luciferase is present together with luciferin in the cold-water extract, but the luciferin is soon oxidized and luciferase alone remains. Mixing a solution of luciferin and luciferase always results in light production until the luciferin is again oxidized. Similar substances have been found by me in the American fireflies, *Photinus* and *Photuris*, the Japanese firefly, *Luciola*, and in the ostracod crustacean, *Cypridina hilgendorfii*. Crozier[1] reports that they exist also in *Ptychodera*, a balanoglossid. I have been unable to demonstrate their existence in luminous bacteria; in the annelid, *Chætopterus;* the pennatulids, *Cavernularia* and *Pennatula;* the

[1] Private communication.

squid, *Watasenia;* and the fish, *Monocentris japonica.* E. B. Harvey (1917) could not demonstrate them in *Noctiluca.* There are several reasons why the existence of such bodies might be difficult to demonstrate, but these reasons cannot be considered here. We thus see that the photogen is in reality of dual nature, that two substances are necessary for light production and that they may be very readily separated because of difference in resistance to heating. In this respect Bioluminescence is similar to some other biological processes, notably to certain immune reactions and to certain enzyme actions.

Thus, for the hæmolysis of foreign red blood corpuscles, a specific immune body (*amboceptor or substance sensibilatrice*) not destroyed by moderate heating, and a thermolable complement (*alexin*) are necessary.

For the alcoholic fermentation of glucose by the zymase of yeast juice two substances are also necessary. The zymase is made up of a heat resistant, dialyzing component, the co-enzyme, and a non-dialyzing substance, destroyed on boiling, the enzyme proper. Both must be present for alcoholic fermentation of glucose to proceed and the two may be separated by dialysis or by their difference in resistance to heating. Several other characteristics of living cells are known to depend on the joint action of two substances, one thermolabile, the other thermostable. The reducing action of tissues, according to Bach, requires a reducing enzyme proper or perhydridase and some easily oxidizable substance, such as an aldehyde. The aldehyde has been spoken of as the co-enzyme.

Because of the necessity of thermostable and thermolable substances for light production in luminous animals and because I was unable to oxidize the thermostable

material of *Cypridina* with such oxidizing agents as $KMnO_4$, H_2O_2, blood and H_2O_2, BaO_2, etc., I called the heat resistant substance of *Cypridina*, "*photophelein*" (from *phos*, light and *opheleo*, to assist), comparable to co-zymase, and the heat sensitive substance of *Cypridina*, "photogenin" (from *phos*, light and *gennao*, to produce), comparable to the zymase proper of yeast. In mode of preparation and properties, the photophelein of *Cypridina* was also comparable to the luciferin of *Pholas* and the *photogenin* of *Cypridina* to the luciferase of *Pholas*. I also regarded photogenin as the source of the light (hence the name), because a solution of *Cypridina* photogenin (=*Pholas* luciferase) will give light on mixing with crystals of salt and other substances which could not possibly be oxidized. I later found, however, that this result was due to the fact that the photogenin solution contained some of the thermostable substance (luciferin) bound (combined or adsorbed), and that this was freed by the salt crystals and oxidized with light production. I have consequently abandoned the view that the system of substances concerned in light production is similar to the zymase—co-zymase system of yeast—and have adopted Dubois' term, luciferase (=*photogenin*) for the thermolable material, and luciferin (=*photophelein*) for the thermostable material.

The luciferin of *Cypridina* differs from that of *Pholas* in that it will not oxidize with light production with any oxidizing agents that I have tried, and will give no light with luciferase from *Pholas*. It does, however, oxidize spontaneously in solution, although no light accompanies this oxidation.

I believe that for accuracy and definiteness we must

designate the luciferins and luciferases from different animals by prefixing the generic name of the animal and speak of *Pholas* luciferin, *Cypridina* luciferase, *Pyrophorus* luciferase, etc. In extracts of many non-luminous animals Dubois has found oxidizing agents which can oxidize *Pholas* luciferin with light production and I have confirmed this for *Pholas,* but I have not found any such substances in non-luminous animals which will oxidize *Cypridina* luciferin with light production. I have found in extracts of non-luminous animals substances which will liberate the bound luciferin in a concentrated *Cypridina* luciferase solution. The luciferin can then be oxidized by the luciferase and light appears. Their effect is similar to that of salt crystals and I suggest that they be called *photopheleins,* substances that assist in the luciferin-luciferase reaction by liberating bound luciferin. One of the best ways of freeing a solution of luciferase from bound luciferin is to shake with chloroform. We can then do away with the disturbing effects of bound luciferin.

It is obvious that luciferin must be formed from some precursor in the cell and following the usual biochemical terminology, Dubois has called it *proluciferin* or *preluciferin,* and believes that it is converted into luciferin by an enzyme co-luciferase. The experiments to prove the existence of proluciferin were first made by Dubois on *Pholas* in 1907 and have since been amplified (1917 *a;* 1918 *a* and *b*).

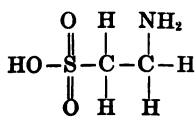
In order to understand these experiments it must be borne in mind that Dubois prepares luciferin from *Pholas* in three ways: (1) By precipitating the viscid luminous fluid from the siphons with 95° alcohol and dissolving the precipitate in water (1901*a,* 1907). (2) By extracting

the luminous organs with 90° alcohol in a closed vessel for twelve hours and filtering (1896). (3) By heating the viscid luminous fluid to 70° C. Apparently *Pholas* luciferin is sparingly soluble in alcohol as it can be obtained either in an alcoholic extract (method 2) or by precipitation with alcohol (method 1). Proluciferin (called *preluciferine* in a later paper, 1917 *a*, 1918 *a*), is prepared by methods 1 or 2 except that fatigued siphons, from which luciferin has been removed by washing, are used (1907, 1917 *a*, 1918 *a*). Preluciferin can also be obtained on boiling an extract of the luminous organ of *Pholas* because luciferin (at 70°), luciferase (at 60°) and a co-luciferase are all destroyed below the boiling point (1917 *a*).

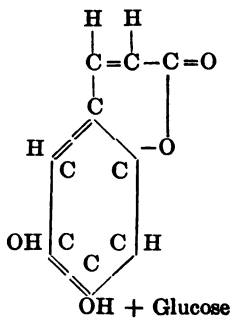
Co-luciferase is prepared (1) by heating a luciferase solution to 65° (1917 *a*) or (2) by extracting with water portions of the siphon of *Pholas* which have previously been macerated and well extracted with alcohol (1918 *a*). Long-continued treatment with alcohol apparently destroys the luciferase without affecting the co-luciferase. On mixing a solution of preluciferin with one of co-luciferase and allowing them to stand for 8–10 hours, luciferase is formed and can be recognized by the fact that it will give light with a crystal of $KMnO_4$. Preluciferin does not do this.

Recently Dubois (1918 *a*) states that preluciferine is nothing but taurine and that taurine occurs in large quantities in *Pholas* and is transformed into luciferine by the action of co-luciferase. Not only taurine, but also Byla's peptone, egg lecithin, and esculin can be converted into luciferine by co-luciferase, and since esculin, a glucoside, is so transformed, Dubois believes this proves that co-

luciferase belongs to the hydrolases. Indeed, it proves too much. Luciferin must have an extraordinary chemical structure if it can be formed by hydrolysis of such diverse compounds as peptone, lecithin, esculin and taurine. A glance at the structural formula of esculin and taurine is sufficient to emphasize the diverse nature of these two substances.



Taurine



Esculin

I believe that in these experiments Dubois has been working with an oxidation product of luciferin, what I have called *oxyluciferin,* rather than a pro-substance. The mode of preparation of *Pholas* preluciferin and *Pholas* co-luciferase is such as could be used in the preparation of *Cypridina* oxyluciferin, and it seems more logical to look for the presence of *Pholas* oxyluciferin in one or both of Dubois' extracts rather than believe that luciferin can be formed from both taurine and esculin. When the co-luciferase solution stands with the preluciferin solution we would in reality have not the formation of luciferin from preluciferin, but the formation of luciferin from oxyluciferin, by some reducing agent in the mixture. Indeed, in a very recent paper Dubois (1919 *c*) takes the

view that his co-luciferase is a reducing enzyme which forms luciferin by reduction (presumably from oxidized luciferin) and no mention is made of preluciferin.

It is, of course, obvious that when luciferin oxidizes, some oxidation products must be formed. Most observers have assumed the oxidation products of luciferin to be relatively simple and to represent a rather complete breaking down of the luciferin molecule. Carbon dioxide was mentioned by Phipson (1872) as being formed. We have just seen that no carbon dioxide is formed during the oxidation of *Cypridina* luciferin and there is evidence that no fundamental change at all occurs. It is for this reason that I have called the oxidation product of luciferin *oxyluciferin.** As we shall later see, the change luciferin oxyluciferin is to be compared to the oxidation of colorless dyes (leuco-compounds) to the colored dye. The chemical properties of oxyluciferin are similar to those of luciferin and the oxyluciferin can be readily reduced to luciferin again.

Finally, we have the fluorescent substance of *Pyrophorus* and fireflies, which Dubois first called *pyrophorin,*

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* It is unfortunate that Dubois (1918 b) has used the term oxyluciferine in a quite different sense from the present use. He regards oxyluciferine as a substance still capable of giving light by autoöxidation, and represents the steps in luminescence as follows:

" Co-luciférase + preluciférine = luciférine.
Luciférase + luciférine = oxyluciférine.
Oxyluciférine + oxygéne = lumiére."

I should represent them as follows:

Luciferin + oxygen $\leftrightarrows$ oxyluciferin.

The reaction proceeds to right with light production only in presence of luciferase.

but later, adopting McDermott's terminology, speaks of as *luciferesceine.* This Dubois regards as a substance intensifying the light and modifying its color by changing invisible into visible rays. As we have seen, this theory, while attractive, will not stand the test of critical examination.

Phipson's noctilucin, while the first name for the photogen of luminous animals, is too vague a substance, chemically, to warrant a retention of the term. Of the names, luciferin, luciferase, preluciferin or proluciferin, coluciferase, photogenin, photophelein, oxyluciferin, luciferesceine, I believe that only proluciferin, luciferin, oxyluciferin, luciferase and photophelein stand for substances which are really significant for the theory of light production. *Luciferin* is the heat resistant, dialyzable substance which takes up oxygen and oxidizes with light production in the presence of the heat sensitive, non-dialyzing, enzyme-like *luciferase.* The luciferin must come from some precursor, *proluciferin,* but I have been unable to demonstrate the existence of this body in *Cypridina* and know nothing definite of its properties. The luciferin oxidizes to *oxyluciferin* which has the same chemical properties as the luciferin itself and may be reduced to luciferin again by reducing substances in luminous and other animals or by inorganic reducing agents. *Photophelein* is a name for substances in various animal or plant extracts which are capable of liberating luciferin from some bound condition in solutions containing luciferase. Under this term are included a number of unknown, probably quite different substances, some of which are thermostable and others thermolabile.

We have seen that Bioluminescence is an oxylumines-

cence, that the light is probably due to the oxidation of a compound, luciferin, in presence of air and water and that the oxidation is accelerated by an enzyme-like substance, luciferase. We also saw in Chapter 2 that light production is of fairly common occurrence during the oxidation of many organic compounds, provided the oxidation is carried out in the proper way. Many of these organic compounds must be oxidized by relatively strong alkali or such strong oxidizing agents as would have a very deleterious action on living cells. In 1913, Ville and Derrien, in a short note to the French Academy, "Catalyse Biochemique d'une Oxydation Luminescente," show that *lophin* could be oxidized by vertebrate blood in the presence and H_2O_3?. In the same year Dubois (1913) found that esculin, the glucoside from horse chestnut bark, would also oxidize and luminesce in presence of blood and H_2O_2. In these cases the hæmoglobin of the blood acts as a catalyst, transferring oxygen from the H_2O_2 to esculin or lophin and is to be compared to luciferase, except that luciferase does not require the presence of H_2O_2.

As the hæmoglobin does not lose this power on boiling, whereas luciferase does, the analogy is far from perfect. Many oxygen carriers are known, however, which may be destroyed on boiling their solutions, namely, the peroxidases of plant juices. Esculin will not luminesce with peroxidase and H_2O_2, but pyrogallol or gallic acid will. If one mixes a test tube containing pyrogallol solution + H_2O_2 with potato or turnip juice or almost any plant extract, a yellowish luminescence appears. The plant extract loses the power to cause such luminescence on boiling and the peroxidase will not dialyze. It is, of course, comparable to luciferase and acts on the thermostable, dialyz-

able pyrogallol – H_2O_2 mixture, which is comparable to luciferin. Curiously enough, although many hydroxy-phenol and amino-phenol compounds can be oxidized by peroxidase and H_2O_2, only pyrogallol and gallic acid will oxidize with light production. Many other oxidizers can take the place of the peroxidase. A list of these is given on page 151. No other peroxide can take the place of H_2O_2 with peroxidases as oxidizers, but a few can replace H_2O_2 with other oxidizers. This is brought out in Table 7.

TABLE 7

Peroxides Giving Light with Pyrogallol and Oxidizers

Oxidizer. (Equal parts added to a mixture of M/100 pyrogallol and the peroxide)	H_2O_2 3 per cent.	Benzoyl hydrogen peroxide (insoluble powder)	Ozonized turpentine (one drop)	Na_2O_2 (powder)	BaO_2 (powder)	MnO_2 (insoluble powder)	PbO_2 (insoluble powder)	K persulfate M/10	Na perborate M/20	K perchlorate M/10	Quinone (insoluble crystals)
Turnip juice	+	–	–	–	–	–	–	–			–
1 per cent blood extract	+	–	–	Faint flash	–	–	–	–	–	–	–
M/20 $K_4Fe(CN)_6$	+	–	–	–	–	–	–	–	–	–	–
M/100 $KMnO_4$	+	–	–	–	–	–	–	Faint flash	Fair flash	–	–
M/10 $FeCl_3$	+								–	–	
M/100 CrO_3	+								–	–	
Na hypobromite	+	–	–	Faint flash	Faint flash	–	–	Fair flash	Fair flash	–	–
Ca hypochlorite	+	–	–	–	–	–	–	Faint	Fair flash	–	–
MnO_2	+										
$Mn(OH)_2$ sol in peptone	+								–	–	
Colloidal Ag	+										

Our knowledge of the existence of such analogous, purely organic chemical oxidations, which proceed with light production, greatly strengthens Dubois' theory that

the luciferin-luciferase reaction really represents a catalytic oxidation of similar nature. As Dubois (1914 *a*) expresses it, we are dealing in luminous organisms with "1° une luminescence; 2° une chemiluminescence; 3° une oxyluminescence; 4° une zymoluminescence.

"Ou si l'on bien admettre que les zymases sont encore quelque chose de vivant, une Biozymoöxyluminescence." Perhaps it is not really necessary to admit that the enzymes are living in order that we may adequately visualize the nature of the photogenic process.

In the next chapter the properties of the three principal substances, luciferin, oxyluciferin and luciferase, will be studied more carefully.

CHAPTER VI

THE CHEMISTRY OF LIGHT PRODUCTION, PART II

SINCE Radziszewski's experiments on the oxidation of oils in alcoholic solutions of alkali, most of the early workers on Bioluminescence tacitly assumed that the oxidizable material was fat or a fat-like substance. Support was given to this view by the occurrence in cells of granules or globules from which the light was seen to come. We now know that these bodies are not fat droplets and that neither luciferin nor luciferase are soluble in such fat solvents as ether, chloroform, benzol or benzine. Phipson's description of the properties of noctilucin are too crude and inaccurate to be considered. Dubois did not study the chemical properties of luciferin and luciferase from *Pyrophorus,* the first form with which he worked, except to point out that *Pyrophorus* luciferase was destroyed on heating and was precipitated by alcohol while the *Pyrophorus* luciferin was not so affected. Luciferin was found only in the luminous organ of *Pyrophorus,* not in the blood; luciferase probably exists throughout the animal.[1]

PHOLAS LUCIFERIN.—In a series of papers since 1887 Dubois has studied the chemical properties of *Pholas* luciferin and *Pholas* luciferase. He finds the luciferin to be destroyed above 70° C., to dialyze slowly, to oxidize with light production in the presence of *Pholas* luciferase, $KMnO_4$, H_2O_2, hæmatine and H_2O_2, BaO_2, PbO_2, hypochlorites, and the blood of various marine mollusks and crus-

[1] Private communication from R. Dubois.

tacea. It is insoluble in fat solvents but forms a colloidal solution in water from which it is precipitated unchanged by picric acid, alcohol at 82°, and $(NH_4)_2SO_4$. It is not precipitated by NaCl, $MgSO_4$ or acetic and carbonic acids, except in presence of neutral salts. It forms an insoluble alkali albumin with NH_4OH. Dubois (1887 *a*) stated at one time that it could be crystallized and has spoken of it as belonging to several different classes of substances, proteose, nucleoprotein, albumin. Most recently he describes luciferin as a natural albumin having acid properties. It occurs only in luminous, not in non-luminous animals, and is found in all parts of the mantle, especially the siphons. It does not occur in non-luminous parts of the mollusk. No photographs of luciferin crystals have ever been published.

PHOLAS LUCIFERASE.—*Pholas* luciferase has all the properties of an enzyme, is destroyed at 60° C., is non-dialyzable, insoluble in fat solvents, but forms a colloidal solution in water. It is not affected by 1 per cent. NaF but its activity is suspended in saturated salt solutions, sugar or glycerine, and it may be preserved in this way, its activity returning on dilution. It is digested by trypsin and slowly destroyed by the fat solvent anæsthetics, such as chloroform. For this reason Dubois regards it as an oxidizing enzyme similar to the oxydones of Batelli and Stern. Because he found iron in an extract of *Pholas* dialyzed for a long time against running water, Dubois considers that it is associated with iron, and reports that it will oxidize the ordinary oxidase reagents, such as pyrogallol, gum guaiac, a-naphthol and para-phenylenediamine, etc. It remains to be proved, however, that luciferase and not the oxidizing systems such as occur in

all cells are responsible for the coloration of these reagents. Dubois has found luciferases or substances capable of giving light with *Pholas* luciferin in the blood of many non-luminous crustacea and mollusks (in *Barnea candida, Solen, Cardium edulis, Ostræa* and *Mytilus*).

CYPRIDINA LUCIFERIN.—Despite the large amount that has been written on luminous animals, Dubois' work on *Pholas* and my own on *Cypridina* and the firefly are the only truly chemical studies that give us any idea of the nature of the photogenic substances in any luminous animal. In many ways *Cypridina* luciferin is similar to *Pholas* luciferin, but the two differ in a sufficient number of points to make certain that they are not identical substances. As I have emphasized above, we should speak not of luciferin and luciferase but of the *luciferins* and the *luciferases*. The luciferins, as the oxidizible substances, must claim first attention. They are more simple substances than the luciferases. If we are to produce light artificially in the same way that animals do, the luciferins must be synthesized. The luciferin of *Pholas* will luminesce with $KMnO_4$ and other oxidizing agents, and, although I have not yet succeeded in oxidizing *Cypridina* luciferin with oxidizing agents, I have no doubt but that some inorganic catalyzer will be found to take the place of luciferase and accelerate oxidation of *Cypridina* luciferin with light production.

The most remarkable peculiarity of *Cypridina* luciferin is its stability. In my first paper on *Cypridina* I stated that luciferin was not destroyed by momentary boiling but would be destroyed if boiled four or five minutes; also, that it was unstable at room temperatures and would disappear from solution in the course or a day or so. The

reason for this is that luciferin oxidizes even in absence of luciferase and will then no longer give light with luciferase. This spontaneous oxidation, which occurs without light production, can be prevented by keeping the luciferin in a hydrogen atmosphere or by the addition of acid. Under these conditions the luciferin can be boiled without destruction or preserved for months without deterioration. The rapid disappearance of luciferin from neutral or alkaline solution on boiling in the air is entirely due to the more rapid oxidation at the boiling point. As the oxidation product, oxyluciferin, can be readily reconverted into luciferin again, we can not consider luciferin unstable in the sense that its molecule is actually destroyed as is the case when luciferase is boiled.

Not only is luciferin stable on boiling but it will actually withstand boiling for 10 hours with 20 per cent. HCl (by weight, sp. gr. = 1.1) or with 4 per cent. H_2SO_4. After one day of boiling with 20 per cent. HCl the luciferin was completely destroyed and with 4 per cent. H_2SO_4 destruction was almost complete. In these cases there was no question of a mere oxidation to oxyluciferin, as no oxyluciferin could be demonstrated after boiling with such strong acids. An actual destruction, probably an hydrolysis of the luciferin molecule, occurred. We shall have occasion to refer to this again in considering the protein nature of luciferin. It must be borne in mind that many proteins require four or five days' boiling with 20 per cent. HCl for complete hydrolysis to amino-acids. Luciferin forms a solution in water, probably colloidal, although the luciferin will dialyze through parchment or collodion membranes. It is rather readily adsorbed by various finely divided materials such as bone black,

$Fe(OH)_3$, kaolin, talc and $CaCo_3$. It is not destroyed by any of the enzyme solutions which I have tried. These include such as are widely divergent in action: pepsin HCl, trypsin, erepsin, salivary and malt diastase, yeast invertase, urease, rennin and the enzymes of dried spleen, kidney and liver substances.

By extracting the dried Cypridinas ground to a powder, the solubility of luciferin in non-aqueous solvents could be easily studied, and by adding such reagents as dilute acids, alkalies, neutral salts and the alkaloidal reagents to an aqueous solution of luciferin the general biochemical behavior of luciferin can be quite accurately stated. For convenience the results of this study are given in Table 8.

Because the luciferin is almost completely precipitated by saturation with $(NH_4)_2SO_4$, we may conclude that it occurs in water in the colloidal state. This excludes it from belonging to one of the numerous groups of biochemical compounds occurring in true solution and places it among the known groups of colloidal substances, the soaps, proteins, polysaccharides, phospholipins, galactolipins (*cerebrosides*), tannins or saponins. It is not a polysaccharide because nearly completely precipitated by phosphotungstic acid, nor a soap because not precipitated by calcium salts, nor a phospho- or galactolipin because insoluble in benzine, hot or cold. It gives no tannin or saponin tests. Only the protein group remains, and of the eighteen protein classes recognized by the American Society of Biochemists, the general properties of luciferin indicate that it should be placed among the natural proteoses, somewhere on the borderland between the proteoses and peptones. The fact that luciferin will dialyze,

TABLE 8

Properties of Photogenic Substances from Cypridina

Property	Luciferase	Luciferin
Salting out		
By saturation NaCl	Not precipitated	Not precipitated.
By half saturation $MgSO_4$	Do.	Do.
By saturation $MgSO_4$	Nearly completely precipitated	Partially precipitated.
By saturation $MgSO_4$+acetic acid	.....	Do.
By half saturation $(NH_4)_2SO_4$	Slightly precipitated	Not precipitated.
By saturation $(NH_4)_2SO_4$	Completely precipitated	Nearly completely precipitated.
By saturation $(NH_4)_2SO_4$+acetic acid	.....	Nearly completely precipitated.
Solubility in		
Methyl alcohol	Insoluble	Soluble.
Ethyl alcohol	Do.	Do.
90 per cent.	Do.	Do.
70 per cent.	Do.	Do.
50 per cent.	Slightly soluble	Do.
Propyl alcohol	Insoluble	Do.
Isobutyl alcohol	Do.	Fairly soluble.
Amyl alcohol	Do.	Slightly soluble.
Benzyl alcohol	Do.	Soluble.
Acetone	Do.	Fairly soluble.
90 per cent	Do.	Soluble.
70 per cent	Slightly soluble	Do.
50 per cent	Fairly soluble	Do.
Ethyl acetate	Insoluble	Do.
Ethyl propionate	Do.	Fairly soluble.
Ethyl butyrate	Do.	Do.
Ethyl valerate	Do.	Slightly soluble.
Ethyl nitrate	Do.	Very slightly soluble.
Glycerine	Do.	Soluble.
Glycol	Do.	Do.
Ether	Do.	Insoluble.
Chloroform	Do.	Do.
Carbon disulfide	Do.	Do.
Carbon tetrachloride	Do.	Do.
Benzol	Do.	Do.
Toluol	Do.	Do.
Xylol	Do.	Do.
Petroleum ether	Do.	Do.
Anilin	Do.	Do.
Glacial acetic acid	Do.	Fairly soluble.

Properties of Photogenic Substances from Cypridina—Continued

Property	Luciferase	Luciferin
Alkaloidal Reagents		
Phosphotungstic acid	Completely precipitated	Very nearly completely precipitated.
Phosphotungstic and acetic acids	.....	Very nearly completely precipitated.
Phosphotungstic acid and HCl	.....	Completely precipitated.
Tannic acid	Nearly completely precipitated	Nearly completely precipitated.
Tannic and acetic acids	.....	Nearly completely precipitated.
Tannic acid and HCl	.....	Nearly completely precipitated.
Picric acid	Nearly completely precipitated	Not precipitated.
Picric and acetic acid	.....	Do.
Picric acid and HCl	.....	Do.
$K_4Fe(CN)_6$ and acetic acid	.....	Do.
Heavy Metal Salts		
Basic lead acetate	Completely precipitated	Not completely precipitated.
Neutral lead acetate	Nearly completely precipitated	Not completely precipitated.
Neutral lead acetate and acetic acid	.....	Not precipitated.
Mercuric chloride	Not precipitated	Not completely precipitated.
Mercuric chloride and acetic acid	.....	Almost completely precipitated.
Uranyl nitrate and acetic acid	.....	Not completely precipitated.
Acids and Alkalies		
NaOH	Not precipitated	Not precipitated.
NH_4OH	Do.	Do.
Acetic acid	Do.	Do.
H_2CO_3	Do.	Do.
Trichloracetic acid	Do.	Do.

although almost completely salted out by $(NH_4)_2SO_4$, is strong evidence in favor of placing it in such a position.

On the other hand, luciferin has two properties which to say the least are unusual for proteins. I refer to its

solubility in alcohols, acetone, esters, etc., and non-digestibility by trypsin or erepsin, which have almost universal proteolytic power.

The best known class of proteins soluble in alcohol is the prolamines of plants, but the prolamines are insoluble in water and in absolute alcohol. Zein, the prolamine of corn, is soluble in 90 per cent. ethyl, methyl, and propyl alcohols, in glycerol heated to 150° C., and in glacial acetic acid. Recently Osborne and Wakeman (1918) have described a protein from milk having solubilities similar to those of gliadin, the prolamine of wheat. Welker (1912) has described a substance, obtained from Witte's peptone, giving the biuret, Millon, and Hopkins-Cole tests, which is soluble in water and absolute alcohol but not in ether, and it is possible that others of the peptones are soluble in absolute alcohol. On the other hand, some proteins in the absence of salts form colloidal solutions in strong alcohol from which they may be precipitated by an appropriate salt. As the absolute alcohol extract of *Cypridinæ* was made from dry material containing the salts of sea water, some salt was present, but there is always the possibility of sol formation.

If we extract dried *Cypridinæ,* which have previously been thoroughly extracted with benzine or ether, with 800 c.c. of boiling absolute alcohol for an hour, filter the alcohol extract through blotting paper and hardened filter paper, quickly evaporate the filtrate to dryness on the water bath, and dissolve the residue in a small quantity of water saturated with CO_2,* we obtain a yellow opalescent solution which gives a bright light with luciferase. This solution contains some protein

* To make the solution slightly acid and prevent oxidation of the luciferin.

after four 1 c.c. additions, no more light is produced. The luciferase is therefore used up and cannot oxidize more than a certain quantity of luciferin. In this experiment, however, we added a concentration of luciferin from one *Cypridina* 100 times that of the luciferase from one *Cypridina,* i.e., four additions each 25 times as concentrated. We have, of course, no way of telling what the absolute amount (in milligrams) of luciferin or luciferase is in a single *Cypridina,* but we do know that the luciferase from one *Cypridina* cannot oxidize luciferin from more than 100 Cypridinas. If the ratio of luciferin to luciferase in a single animal is 100:1, it would mean that luciferase could oxidize 10,000 times its weight of luciferin. A large excess of luciferin but not an indefinite quantity can be oxidized by luciferase, and I believe this is sufficient justification for considering luciferase an enzyme, although it is not an ideal example of an organic catalyzer. Quite a number of enzymes are known to be diminished during the course of the reaction they accelerate or to be poisoned by their reaction products. Enzyme reactions inhibited by the formation of reaction products again proceed if these are removed or diluted. However, light does not again appear in a mixture of weak luciferase with excess of luciferin upon dilution with water, so that the luciferase cannot have been merely inhibited by some reaction product but must have been actually used up during the reaction. It should be noted in passing that the peroxidases, ordinarily spoken of as oxidizing enzymes, are used up in the reaction and can only oxidize limited amounts of oxidizable substances, a quantity almost in proportion to the concentration of peroxidase present.

Whether luciferase is an oxidizing enzyme made up of

an albumin associated with some heavy metal as iron, copper or manganese is uncertain. From analyses of whole *Cypridina,* kindly made for me by Prof. A. H. Phillips of Princeton University, all three of these metals, which we know to be associated with biological oxidations, are present, and it is quite possible that one of them is concerned with the oxidation of luciferin.

Although I have tested a great many oxidizers, organic and inorganic, and a large number of oxidizing enzymes from blood and tissue extracts of animals rich in iron, copper and manganese, I have found no material which is capable of taking the place of *Cypridina* luciferase. Peroxidases or oxidases of plants, hæmoglobin, hæmocyanin, extracts of mussels, manganese containing blood of various marine crustacea and mollusks will give no light on mixing with luciferin. Such active oxidizers as $KMnO_4$, H_2O_2, BaO_2, and many others, will not oxidize *Cypridina* luciferin with light production, although they can oxidize *Pholas* luciferin with light production.

The action of *Cypridina* luciferase is very highly specific. It is found only in the luminous organ of *Cypridina hilgendorfii,* not in non-luminous parts and not in a non-luminous species of *Cypridina* closely related to *hilgendorfii.*

Luciferins and luciferases from closely allied luminous forms will mutually interact to produce light, but no light appears if these substances come from distantly related forms. Thus firefly (*Photuris*) luciferin will give light with *Pyrophorus* luciferase and *vice versa,* but *Cypridina* luciferin will give no light with firefly (*Luciola*) luciferase or *vice versa,* nor with *Pholas* luciferase or *vice versa.* The faint luminescences sometimes observed on mixing

firefly or *Cypridina* luciferase with boiled extracts of non-luminous forms, or of distantly related luminous forms, are probably caused by photophelein in the boiled extract.

Like the plant peroxidases, *Cypridina* luciferase is not readily affected by the action of chloroform, toluol, etc. Unlike the plant peroxidases, it will not oxidize (*i.e.*, produce coloration) in either presence or absence of H_2O_2, any of the hydroxyphenol or aminophenol compounds, such as pyrogallol, a-naphthol, para-diamino-benzine, gum guaiac, etc., commonly used as peroxidase reagents. Neither will luciferase produce light with any substances, such as oils, lophin, pyrogallol, gallic acid, esculin, etc., which we know to be capable of oxidation with light production by other means. The luciferases are very highly specific and act only upon the luciferins of the same or closely related species. They must be placed by themselves in a new class of oxidizing enzymes.

According to Dubois, *Pholas* luciferase is rather readily destroyed by chloroform and my own observations indicate that this is true also of firefly luciferase, so that a certain amount of variation exists in the group of luciferases.

None of the luminescent animals which I have studied are at all affected by cyanides. The luminescence continues in extracts of *Cypridina*, firefly, and *Cavernularia*, or in *Noctiluca* and luminous bacteria after addition of small or high ($m/40$) concentrations of KCN. In this respect the luciferases are very different from many types of oxidizing enzymes which are inhibited by exceedingly weak concentrations of cyanide. It should be borne in mind, however, that while KCN inhibits catalase and the

catalytic decomposition of H_2O_2 by Pt or Ag, it does not affect the catalytic decomposition of H_2O_2 by thallium.

OXYLUCIFERIN.—When luciferin is oxidized it must be converted into some substance or substances and I believe this change involves no fundamental destruction of the luciferin molecule as it is a reversible process. I shall speak of the principal (if not the only) product formed as *oxyluciferin.*

If we assume that the oxidation of luciferin changes the molecule but slightly, we at once think of comparing the change luciferin $\rightleftarrows$ oxyluciferin with the change reduced hæmoglobin $\rightleftarrows$ oxyhæmoglobin. The condition is, however, not so simple as this, for oxyhæmoglobin will again give up its oxygen providing the partial pressure of oxygen is made sufficiently low, whereas oxyluciferin will not do this, at least in the dark. We can not reduce oxyluciferin solution by exhausting the oxygen with an air-pump.

There is another oxidation-reduction system which can also be easily reversed, but not by merely removing the oxygen from the solution—that is, the reduction of a dye such as methylene blue to its leuco-base. I believe the change which occurs when luciferin is oxidized is similar to that which occurs when the leuco-base of methylene blue or sodium indigo-sulphonate is oxidized to the blue dye. Oxidation of leuco-dye bases occurs spontaneously in presence of oxygen and appears to consist in the removal of hydrogen from the leuco-base with formation of water. Reduction of these dyes may be effected in the same ways that oxyluciferin can be reduced. In the case of methylene blue, reduction consists in the addition of two hydrogen atoms. Whether a similar change occurs

when oxyluciferin is reduced or whether oxygen is actually added as in formation of hæmoglobin cannot be definitely stated at present. We may write equations representing these possibilities as follows:

$$C_{16}H_{20}N_3SCl \text{ (leuco-methylene blue)} + O \leftrightarrows C_{16}H_{18}N_3SCl \text{ (methylene blue)} + H_2O$$

$$\text{Hæmoglobin} + O \leftrightarrows \text{oxyhæmoglobin.}$$

Let us now turn to the methods which may be used in reduction of oxyluciferin. We may then endeavor to write an equation which will represent the fundamental changes in the luminescence reaction.

My attempts to reduce the oxidation product of luciferin started from the observation that if one places a clear solution of luciferase in a tall test tube, although it may give off no light at first when shaken, after standing a day or so a very bright light would appear on shaking. This was especially true when the luciferase had become turbid and ill-smelling from the growth of bacteria. Thinking that the bacteria produced a substance which could be oxidized by the luciferase, I tried growing bacteria and also yeast on appropriate culture media, and after some days of growth mixing the culture media containing the products of bacterial or yeast growth with luciferase, expecting to obtain light; but no light appeared. However, if a little crude luciferase solution was added to the bacterial or yeast cultures and then allowed to stand for some hours, light appeared whenever they were shaken. Indeed such cultures behaved much as a suspension of luminous bacteria which has used up all the oxygen in the culture fluid and will only luminesce when, by shaking, more oxygen dissolves in the culture medium. Real-

izing that in bacterial cultures in test tubes, anaërobic conditions soon appear, and also the strong reducing action of bacteria upon many substances (for instance, nitrates or methylene blue) under anaërobic conditions, it struck me that the bacteria might be reducing the oxidation product of luciferin to luciferin again. We must remember that since crude luciferase solution is a cold-water extract of a luminous animal allowed to stand until all the luciferin has been oxidized, it must contain oxyluciferin as well as luciferase and will give light if the oxyluciferin is again reduced and oxygen admitted. This appears to be the correct explanation of the above experiments.

Oxyluciferin may also be readily reduced by the use of the blood of the horse-shoe crab (*Limulus*) allowed to stand until bacteria develop. This experiment is of special interest because the blood contains hæmocyanin, which is colorless in the reduced condition and blue in the oxy-condition. The color change thus serves as an indicator of the oxygen concentration in the blood. A sample of foul-smelling *Limulus* blood full of bacteria will become colorless on standing in a test tube for 10 to 15 minutes, but the blue color quickly returns if shaken with air. Such a blood has the power of reducing oxyluciferin through the activity of the bacteria which it contains. Fresh blood has very little if any reducing action.

Not only bacteria but also tissue extracts have a strong reducing action in absence of oxygen. Thus, muscle tissue stained in methylene blue will very quickly decolorize (reduce) the methylene blue if oxygen (air) is kept away, but the blue color immediately returns if air is admitted. Oxyluciferin (*i.e.*, a solution of luciferin which has been

completely oxidized by boiling or standing in air until it no longer gives light with luciferase) if mixed with a suspension of ground frog's muscle and kept in a well-filled and stoppered test tube for some hours, is reduced to luciferin and gives a bright light if now poured into luciferase solution. Frog muscle suspension alone, or oxyluciferin alone, give no light with luciferase, nor will a mixture of frog muscle suspension and oxyluciferin, if shaken with air for several hours. Only if this last mixture be kept under anaërobic conditions is the oxyluciferin reduced.

The reducing action of tissues is said to be due to a reducing enzyme (*reducase* or *reductase*), itself composed of a perhydridase and some easily oxidized body such as an aldehyde. In the presence of the perhydridase the oxygen of water oxidizes the aldehyde and the hydrogen set free reduces any easily reducible substance which may be present. There is a perhydridase in fresh milk, spoken of as *Schardinger's enzyme,* which is destroyed by boiling. If some aldehyde is added, fresh milk will reduce methylene blue to its leuco-base or nitrates to nitrites, upon standing a short time. If shaken with air the blue color returns. There is no reduction unless an aldehyde is added or unless some boiled extract of a tissue such as liver is added. The boiled-liver extract has no reducing action of its own, but supplies a substance similar to the aldehyde which has been spoken of as *co-enzyme.* The aldehyde is oxidized to its corresponding acid. Milk will reduce methylene blue without aldehyde if bacteria are present in large numbers. There is no reduction if the milk, methylene blue, and aldehyde are agitated with air. The temperature optimum is rather high, 60° to 70° C.

I find that milk is a favorable and convenient medium for the reduction of oxyluciferin and that it acts without the addition of an aldehyde or the presence of bacteria. There is probably a substance acting as the aldehyde in the luciferase-oxyluciferin solution. No light appears if milk is added to a luciferase-oxyluciferin solution, but if the mixture is allowed to stand in absence of oxygen light will appear when air is admitted. The air can be conveniently kept out by filling small test tubes completely with the solution and closing them with rubber stoppers.

As almost all animal tissues contain reductases it is not surprising to find that a freshly prepared and filtered extract of *Cypridina* containing oxyluciferin and luciferase, which gives no light on shaking, will, on standing in a stoppered tube for 24 hours at room temperature in the dark give light when air is admitted. While this may be due to the development of bacteria with a reducing action, it does not seem likely, as under the same conditions methylene blue is not reduced in 24 hours, and there is no turbidity or smell of decomposition in the tube. In 48 hours bacteria appear and methylene blue is also reduced. If we add chloroform, toluol or thymol to the tubes of *Cypridina* extract to prevent the growth of bacteria, and allow them to stand 48 hours, upon admitting air the tube with chloroform gives no light but the tubes with toluol and thymol do give light, although it is not so bright as if they were absent. I believe that these substances have a destructive action on the reductases, most complete in the case of chloroform. Dubois (1919 *c*) also has recorded the occurrence of a reducing enzyme in *Pholas,* a "hydrogenase," which is able to form hydrogen from cane sugar, and luciferin from a boiled ex-

or Al is placed in water. With Mg the water becomes only slightly alkaline from formation of almost insoluble $Mg(OH)_2$. If we add some Al powder and dilute NaOH to an oxyluciferin solution, H is given off and luciferin is formed. As oxyluciferin cannot be formed by the addition of alkali alone we must have in this experiment a reduction of oxyluciferin in alkaline medium by the nascent H produced. Luciferin can also be formed by merely adding Al or Zn or Mg dust to an oxyluciferin solution. Methylene blue can also be readily reduced to its leucobase by Zn dust or Al + NaOH.

Indeed, if one adds some Al or Zn or Mg powder to a solution of luciferase, light will appear whenever the solution is shaken. Luciferase solution must always contain the oxidation product of luciferin, oxyluciferin. In presence of nascent H this is reduced to luciferin, and since the reaction of the medium is alkaline and luciferase is present this is oxidized with light production, when, by shaking, air is dissolved. The light can never become very bright except at the surface because of the deficiency of oxygen in the solution. It would seem, then, that the action of bacteria, yeast, muscle cells, etc., on oxyluciferin must be due not entirely to their acid reaction but to their reducing power as well.

The above experiment is a very striking and instructive one. Given a test tube of luciferase solution containing, as it does, oxyluciferin, add some Zn dust or Mg powder, and the evolution of hydrogen begins. Conditions are now favorable for the reduction of oxyluciferin and this occurs. Shake the contents of the tube to dissolve oxygen and light appears. Allow the tube to stand and the light soon disappears. Shake again and the light reappears. The lumi-

nescence reduction and oxidation process can be demonstrated many times.

A similar experiment can be performed with luciferase and oxyluciferin solution by addition of NH_4SH. This will serve also as another example of the reduction of oxyluciferin in an alkaline medium. Whenever we shake a tube of luciferase, oxyluciferin and NH_4SH, light will appear. When the tube is at rest it becomes dark. Even the merest touch is sufficient to agitate the tube contents, cause solution of oxygen and appearance of light. It is just as if we stimulate the tube to produce light and I believe the phenomenon has a deeper significance and a more fundamental similarity to the phenomena of stimulation than may at first appear. What more simple means of controlling a process can we think of than by admission or withdrawal of oxygen? The firefly turns on its light by stimulation through nerves of the luminous organ. *Noctiluca* flashes on stimulation of any kind, even the slightest agitation causing a brilliant emission of light. If the stimulation process means merely the admission of oxygen to the photogenic cells we have a mechanism in the cell itself for automatically producing the light. The admission of oxygen results in aërobic conditions and luciferin in presence of luciferase can then oxidize to oxyluciferin with luminescence. When the oxygen is used up, the light ceases, anaërobic conditions prevail, and the oxyluciferin is reduced to luciferin again. Thus, luciferin is reformed during the rest period of *Noctiluca* or between the flashes of the firefly. What more efficient type of light than this is to be desired?

Again, methylene blue offers an interesting parallel to oxyluciferin. A little NH_4SH added to methylene blue

solution will reduce (decolorize) it to the leuco-base. If the tube is now shaken the blue color returns. On standing reduction again occurs. The process can be repeated a number of times, the reaction going in one or the other direction, depending on the oxygen content of the mixture.

As methylene blue contains no oxygen, its reduction consists in the addition of two atoms of hydrogen. When leuco-methylene blue oxidizes, water is formed by the union of these two atoms of hydrogen with oxygen, thus:

$$\underset{\text{(leuco-methylene blue)}}{C_{16}H_{20}N_3SCl} + O \leftrightarrows \underset{\text{(methylene blue)}}{C_{16}H_{18}N_3SCl} + H_2O$$

Briefly – $MH_2 + O \leftrightarrows M + H_2O$

To reduce methylene blue we can add the two hydrogen atoms directly from nascent hydrogen formed in the solution or we can split up water by a catalyzer in the presence of some substance, which will take up the oxygen of water, thus:

$$\underset{\text{(Sodium hypophosphite)}}{Na\,H_2\,PO_2} + H_2O + Pd = \underset{\text{(Sodium phosphite)}}{Na\,H_2\,PO_3} + H_2 + Pd$$

This reaction occurs in presence of finely divided palladium. Methylene blue can be reduced by the H_2 and the hypophosphite oxidized.

Since oxyluciferin can be reduced by palladium and sodium hypophosphite (Harvey, 1918), it is probable that we can write the equation for reduction of oxyluciferin and oxidation of luciferin in a similar manner to that of methylene blue:

$$\text{Luciferin} + O \leftrightarrows \text{Oxyluciferin} + H_2O$$

Briefly – $LH_2 + O \leftrightarrows L + H_2O$.

Just as in the case of methylene blue the reaction proceeds in the right hand direction spontaneously if the

pressure of 0 is sufficiently high. If luciferase is also present we have luminescence.

$$LH_2 + O + \text{luciferase} \leftrightarrows L + H_2O + \text{luciferase (luminescence)}$$

The reaction proceeds in the left hand direction under low oxygen pressure, in the presence of nascent hydrogen or with some catalyzer which is able to split water, transferring the H_2 to oxyluciferin and the O to an acceptor (A). NaH_2PO_2 plays the part of the acceptor.

$$L + H_2O + A + Pd = LH_2 + AO + Pd.$$

This appears to be the way in which the reducing enzymes or perhydridases (comparable to the Pd) of living tissues act (Bach, 1911–13) and the action of yeast cells, bacteria, muscle suspensions, etc., in reducing oxyluciferin must occur in the same manner.

If we assume that the LH_2 (luciferin) compound is dissociated to even the slightest extent into L and hydrogen, the hydrogen ion will shift the equilibrium toward the formation of that substance which involves the taking up of hydrogen. Consequently we may obtain a partial formation of luciferin by adding an acid to oxyluciferin. Reduction of the H-ion concentration tends to shift the equilibrium in the opposite direction. Consequently, addition of alkali favors the oxidation of luciferin, and it is quite generally true that biological oxidations are favored by an alkaline reaction. In addition oxygen in alkaline medium has a higher oxidation potential than in neutral or acid media. I believe this is the explanation of the action of acid in formation of luciferin from oxyluciferin.

Addition of acid is not the only means of favoring the formation of luciferin from oxyluciferin. Any reac-

tion which proceeds in one direction with evolution of light should, theoretically, proceed in the opposite direction under the influence of light. So far as I know the case of a reaction, photogenic in one direction and photochemical in the other direction, has never been described, unless we are to accept the cases of phosphorescence, for instance, the absorption of light by CaS and its emission in the dark. However, the reaction which occurs during phosphorescence cannot be stated.

It is a fact that light will cause the reduction of oxyluciferin. A tube of oxyluciferin exposed to sunlight for six hours, or the mercury arc for two hours, will be partially converted into luciferin. It will luminesce when luciferase is added, while a control tube kept in darkness shows no trace of luciferin. The action is more marked with the ultra-violet as a solution of oxyluciferin in a quartz tube showed more reduction than one in a glass tube when exposed for the same length of time to the quartz mercury arc. The reduction is not dependent on the formation of acid under the influence of light since two tubes of oxyluciferin, one kept in darkness and the other exposed to sunlight for six hours, had the same reaction, $P_H = 9.3$. Of course some reducing substance might be formed under the influence of light but this is not very probable.

We may therefore write the reaction for luminescence in the following way:

$$\text{luciferin } (LH_2) + O \underset{\text{perhydridase}}{\overset{\text{darkness, alkali, luciferase}}{\leftrightarrows}} \text{oxyluciferin } (L) + H_2O \text{ (luminescence)}$$
$$\text{acid, light}$$

Acid and light favor reduction while alkali and darkness favor oxidation in the luciferin $\rightleftarrows$ oxyluciferin reaction. Whether the luciferin be really oxidized by removal of H_2 or whether by addition of oxygen is, of course, uncertain, but the analogy with methylene blue is striking and may serve as a working hypothesis until the composition of luciferin and its oxidation product are known.

While I have not studied the properties of oxyluciferin as fully as those of luciferin, so far as I can judge, both substances give the same general reactions and possess identical properties. Both crude luciferin and crude oxyluciferin solution are yellow in color, but I do not believe that either pure luciferin or oxyluciferin are yellow in color, because an ether or benzine extract of *Cypridina* is also yellow, although luciferase, luciferin, and oxyluciferin are insoluble in ether and benzine. The yellow pigment which can be observed to make up part of the luminous gland of *Cypridina* is not luciferin or luciferase. It may be a pigment related to *urochrome.*

When tests are applied and precipitating reagents are added to crude luciferin and crude oxyluciferin solution, they give identical results in each case. A more complete account of the chemistry of luciferin has been given in this chapter, and there is no need of duplicating these statements regarding oxyluciferin. Like luciferin, the oxyluciferin will pass porcelain filters, dialyze through parchment or collodion membranes, and is undigested by salivary diastase, pepsin HCl, Merck's pancreatin in neutral solution, and erepsin. The salivary diastase and the pancreatin (containing amylopsin, trypsin, and lipase) were allowed to digest for four days at 38° C. without showing any evidence of digestive action.

As luciferin is so easily oxidizable a substance, we should expect to find that it will reduce just as glucose will reduce. However, a concentrated solution of luciferin has no reducing action on Fehling's (alkaline Cu), Barfoed's (acid Cu), Nylander's (alkaline Bi) or Knapp's (alkaline Hg) reagent. Glucose will reduce methylene blue in alkaline (not in neutral solution), but luciferin will not reduce methylene blue in alkaline or neutral solution. It would seem, then, that luciferin must contain no aldehyde group. If so, we should expect to obtain reduction of some of the above reagents. Just what group is concerned in the oxidation is unknown at the present time, and in the absence of more experimental data, speculation regarding it can be of little value.

SUMMARY

In summing up we may say that the luminescence of at least three groups of luminous animals, the beetles, *Pholas,* and *Cypridina,* has been definitely shown to be due to the interaction of two substances, luciferin and luciferase, in presence of water and oxygen. Luciferin and luciferase have quite different properties and may be easily separated from each other by various chemical procedures. As the luciferins and luciferases from different luminous animals have somewhat different properties, they may be designated by prefixing the generic name of the animal from which they are obtained.

Cypridina luciferin differs from *Pholas* luciferin in that it can not be oxidized with light production by $KMnO_4$, H_2O_2, with or without hæmoglobin, or similar oxidizing agents. *Cypridina* luciferase differs from *Pholas* and firefly luciferase in that it is not readily

destroyed by the fat-solvent anæsthetics, such as chloroform, ether, etc.

When *Cypridina* luciferin is oxidized, no fundamental splitting of the molecule occurs, because the product, oxyluciferin, can be readily reduced to luciferin again. This reduction is brought about under conditions similar to those necessary for the reduction of dyes, such as methylene blue. Oxyluciferin can be reduced to luciferin, which will again give light with luciferase, by the reductases of muscle tissue, liver, etc., or by bacteria; by Schardinger's enzyme of milk; by H_2S; by the nascent hydrogen from the action of acetic acid on magnesium or of water or NaOH on aluminium, zinc or magnesium; and by palladium black and sodium hypophosphite, all well-known reducing methods. Reduction of oxyluciferin no doubt occurs even in presence of luciferase if oxygen is absent, and reduction of oxyluciferin no doubt occurs in animals which burn luciferin within the cell, thus tending for conservation of material. Dilute alkali favors oxidation and dilute acid favors the reduction. Light favors the reduction of oxyluciferin.

Apparently luciferin and oxyluciferin have identical chemical properties. Neither is digested by the enzymes: malt diastase, ptyalin, yeast invertase, pepsin, trypsin, steapsin, amylopsin, rennin, erepsin, urease or enzymes occurring in the water extracts of dried spleen, kidney, or liver. Luciferase is destroyed only by pepsin (probably), trypsin, erepsin, and something in spleen and liver extract.

Luciferase is unquestionably a protein and all its properties agree with those of the albumins. Although used up in oxidizing large quantities of luciferin,

it behaves in many ways like an enzyme and may be so regarded.

Luciferin, on the other hand, is not digested by proteolytic enzymes, is dialyzable, almost but not completely precipitated by saturation with $(NH_4)_2SO_4$, and is soluble in absolute alcohol, acetone, and some other organic solvents, but not in the strictly fat-solvents like ether, chloroform, and benzol. There are, however, certain CO-NH linkages which are not attacked by proteolytic enzymes and some peptones soluble in absolute alcohol, so that these two characteristics do not bar it from the group of proteins. Luciferin, in fact, has many properties in common with the proteoses and peptones but its chemical nature cannot be definitely stated at present.

CHAPTER VII

DYNAMICS OF LUMINESCENCE

ONE of the most extraordinary things regarding luminescence in general is the small amount of material necessary to cause a visible emission of light. To take an extreme case, the flash of light resulting from the impact on ZnS of a single α particle, a helium atom, is visible to the naked eye. Addition of one part in a million of some heavy metal to pure CaS will confer phosphorescent properties on the latter. We are forced to believe that the heavy metal enters into some reaction during illumination which is reversed with light emission after illumination and a very small amount of heavy metal is necessary. Pyrogallol in water, 1:5,000,000 (m/512,000), can be oxidized with light production by $K_4Fe(CN)_6$ and H_2O_2 (Harvey, 1917) and m/100 pyrogallol + H_2O_2 will give a visible light with colloidal platinum in 1:250,000 concentration (Goss, 1917).

Luciferin and luciferase from *Cypridina* will also luminesce in exceedingly small concentration. If one grinds a single *Cypridina* in a mortar with water and dilutes the extract to 25,600 c.c., light can be observed if luciferin is added to this dilute luciferase solution. By determining the volume of the luminous gland of *Cypridina* and even assuming that this volume is all luciferase, one can calculate that one part of luciferase in 1,700,000,000 parts of water will give light when luciferin is added. Likewise, a similar dilution of luciferin will give visible light when luciferase is added.

The sensitivity of our eye is largely responsible for the detection of so small an energy change. As we have seen, recent determinations have proved that the dark adapted eye can detect 18×10^{-10} ergs per second. From the heat of complete oxidation of pyrogallol it is possible to calculate the amount of pyrogallol necessary to give 18×10^{-10} ergs if completely oxidized. This quantity is infinitesimally small. When pyrogallol is oxidized by $K_4Fe(CN)_6$ and H_2O_2, it is not completely oxidized and probably only a small amount of the energy is converted into light; otherwise we should be able to see the luminescence of a very much weaker concentration of pyrogallol. As the reaction luciferin $\rightleftarrows$ oxyluciferin is so easily reversible, very little energy must be liberated, and, as experiments indicate, very little heat, if any, accompanies light production. Even though this be true, it is still possible for a very small amount of luciferin to produce a very large amount of light.

A very small amount of luciferase only is necessary because it behaves as an enzyme and follows the general rule that catalysts act in minute concentrations.

On the assumption that luciferase is an enzyme, an organic catalyst oxidizing luciferin with light production, we may appropriately inquire into the relation between the concentration of luciferin and luciferase and intensity and duration of luminescence. Oxygen tension, hydrogen ion concentration and temperature must be maintained constant as these all affect both intensity and duration of luminescence. Before considering luciferin and luciferase, however, let us study a few well-known chemiluminescent oxidations with special reference to concentration of reacting substances and temperature.

The effect of temperature on luminescence is of special interest because it gives us a means of analysis for determining if the luminescence depends on reaction velocity. We know that photochemical reactions are very little affected by temperature because the reaction is dependent on the absorption of light, a physical process, and this increases only a small per cent. for a rise of temperature of 10° C. To put it in the usual way, its temperature coefficient (Q_{10}) for a 10° interval is usually less than 1.1. On the other hand, we should expect photogenic reactions, in which some of the chemical energy is converted into radiant energy, to give off much more light the greater the reaction velocity. As reaction velocity increases so rapidly with temperature ($Q_{10} = 2$ to 3), luminescence intensity should rapidly increase with increase in temperature.

Trautz (1905), from his extensive study of the chemiluminescence of phenol and aldehyde compounds came to the conclusion that luminescence intensity was proportional to reaction velocity. He based his conclusions largely on the effects of temperature and concentration of reacting substances and went so far as to declare that any reaction would produce luminescence if the reaction velocity were sufficiently increased. It is quite true that increasing the temperature does increase the intensity of chemiluminescence, but this is only within certain limits. As we raise the temperature, chemiluminescence becomes more intense but we soon reach a temperature for maximum luminescence and above this the intensity diminishes. This is especially well seen in the action of various oxidizers on pyrogallol and H_2O_2 recorded in Table 10. At 100° C. practically no light is produced by many

TABLE 10

Temperature and Light Production. The Oxidizer is Mixed with an Equal Amount of M/100 *Pyrogallol* + *3 per cent.* H_2O_2

Oxidiser	Temperatures				
	0–2°	20°	50°	75°	98–100°
Turnip juice	Faint	Good	Good		Negative.
1 per cent blood extract	Faint	Fair	Good		Fair.
M/20 $K_4Fe(CN)_6$	Negative	Good	Bright	Bright	Good.
M/100 $KMnO_4$	Fair	Good	Bright	Bright	Faint flash.
M/50 $K_2Cr_2O_7$	Negative	Fair	Faint	Fair	Negative.
M/100 CrO_3	Negative	Good	Bright	Bright	Faint.
M/10 KCr alum	Negative	Faint	Faint	Faint	Negative.
M/10 NH_4Fe ''' alum	Negative	Faint	Faint	Faint	Very faint.
MnO_2	Negative	Fair	Fair	Fair	Negative.
NaClO	Bright flash	Bright flash	Bright flash		Fair flash.

oxidizers which are themselves unaffected at 100°. If we are to connect reaction velocity with intensity of luminescence we must conclude that the evolution of light is dependent rather on an optimum than a maximum reaction velocity.

Quite a number of instances are known in which increasing the mass of reacting substances leads not to an increase but to an actual cessation of luminescence. This fact does not confirm the theory that reaction velocity is a determining factor in luminescence. The conditions for the luminescence of white phosphorus are most interesting and unusual. (See van't Hoff, 1895; Ewan, 1895; Centnerszwer, 1895; Russell, 1903; Scharff, 1908.) Phosphorus will only begin to luminesce at a certain small pressure of oxygen. This "minimum luminescence pressure" of oxygen is very low, so low that earlier observers, failing to remove traces of oxygen, thought that luminescence might occur in absence of oxygen. Curiously enough there is also a "maximum luminescence pressure" of oxygen above which no luminescence occurs. Phosphorus will not luminesce in pure oxygen. Between the minimum and maximum is an "optimum luminescence pressure" where luminescence of the phosphorus is brightest. The exact values of these pressures vary with degree of water vapor present and with temperature. According to Abegg's *Handbuch der anorganischen Chemie,* the maximum luminescence pressure with water vapor present, is 320 mm. Hg at 0° and increases 13.19 mm. Hg for each degree rise in temperature. This means that for a definite temperature, say, 20°, phosphorus will not luminesce with an oxygen pressure of 583 mm. Hg, but will luminesce with pressures under this. If, however, we raise the tempera-

ture, luminescence will occur with an oxygen pressure of 583 mm. Hg.

A somewhat analogous case is presented by the oxidation of pyrogallol solution in contact with ozone, except that in this reaction too high a concentration of pyrogallol will hinder the oxidation. I have not studied the effect of varying concentrations of ozone. If oxygen, passed through an ozonizer (the silent electric discharge tube), is bubbled through m/100 pyrogallol, no luminescence occurs at 0°, a fair luminescence at 20°, a good luminescence at 50°, and a bright luminescence at the boiling point. If the pyrogallol is of *m* concentration, no luminescence occurs at 0° or 20°, a fair luminescence at 50°, and a bright luminescence at the boiling point. For a definite temperature, say 20°, no light appears if the pyrogallol is of *m* concentration, but if we raise the temperature, luminescence can occur. The similarity to phosphorus is obvious. Thus the "maximum luminescence pressure" of pyrogallol increases with increase of temperature.

We have already seen that pyrogallol can also be oxidized, if H_2O_2 is present, by a great variety of substances, such as peroxidases of potato or turnip juice, hæmoglobin, $KMnO_4$, $K_4Fe(CN)_6$, CrO_3, MnO_2, hypochlorites and hypobromites, or colloidal Pt and Ag. For convenience we may collectively speak of these as oxidizers. They are recorded in Table 13. No light occurs if H_2O_2 is absent. In the case of some of these oxidizers pyrogallol will luminesce in dilute concentrations but not in strong. Also, dilute pyrogallol will luminesce with a dilute solution of oxidizer but not with a concentrated solution of oxidizer. The effect of rise in temperature in these cases also is to increase the "maximum luminescence concen-

tration" of pyrogallol and the "maximum luminescence concentration" of oxidizer. Table 11 shows this effect of temperature with $K_4Fe(CN)_6$ and varying concentrations of pyrogallol, and Table 12 shows the effect of temperature with pyrogallol and varying concentrations of $K_4Fe(CN)_6$. Table 10 shows the relation between temperature and intensity of luminescence with pyrogallol and various oxidizers. The terms *faint, fair, good,* and *bright* are purely relative designations of brightness as estimated by the eye, for accurate measurements of weak intensities are very difficult to make.

From Table 10 it should be noted that the intensity of luminescence of pyrogallol oxidized with most oxidizers is actually less at the boiling point, a fact which I have repeatedly verified. Let us now see how these facts are to be explained. If we assume that luminescence is dependent on reaction velocity, the intensity of luminescence should increase with increasing temperature. Up to a certain limit this is what we find, but at temperatures above this limit the intensity of luminescence actually decreases. The duration of luminescence also decreases. There is an optimum temperature for luminescence in many cases and we can only conclude that luminescence depends not on a very rapid reaction velocity but on a certain definite reaction velocity. Assuming that this is true, how can we account for the anomalous fact that in high concentrations of oxygen, phosphorus will not luminesce or that in high concentrations of pyrogallol, there is no luminescence in presence of ozone or of oxidizer and H_2O_2. Of course with high active mass of oxygen (in case of phosphorous luminescence) or of pyrogallol (in case of pyrogallol luminescence) the reaction velocity must be

TABLE 11

Temperature, Concentration of Pyrogallol, and Light Production. An Equal Amount of M/20 $K_4Fe(CN)_6$ is Mixed with Pyrogallol + 3 per Cent H_2O_2

Concentration of pyrogallol (after mixing)	Temperatures						
	0–2°	10°	20°	30°	50°	75°	98–100°
M/4	Negative	Negative	Negative	Very faint	Faint	Fair	Faint
M/40	Negative	Faint	Faint	Faint	Good	Bright	Good
M/400	Faint	Fair	Good	Good	Good	Bright	Bright flash
M/4,000	Bright	Bright	Bright	Bright	Bright flash	Fair flash	Negative

TABLE 12

Temperature, Concentration of Ferrocyanide and Light Production. An Equal Amount of $K_4Fe(CN)_6$ is Mixed with M/100 Pyrogallol + 3 Per Cent H_2O_2

Concentration of $K_4Fe(CN)_6$ exposed to light (after mixing)	Temperatures						
	0–2°	10°	20°	30°	50°	75°	98–100°
Half saturated at 20° C.	Negative	Faint	Fair	Fair	Good	Good	Faint flash
One-sixth saturated at 20° C.	Very faint	Fair	Good	Good	Bright	Very bright	Good flash

TABLE 13

Substances Giving Light with Pyrogallol and Hydrogen Peroxide

	Equal volume added to mixture of 1 part M/100 pyrogallol or 1 part 3 per cent H_2O_2 +1 part M/100 pyrogallol; hence, concentrations in final mixture are one-half that given		Light with pyrogallol	Light with pyrogallol +H_2O_2	Blueing of gum guaiac	Blueing of gum guaiac +H_2O_2	Liberation of oxygen from H_2O_2
1	Potassium ferrocyanide	($K_4Fe(CN)_6$ M/10–M/20)	–	Bright	+		+
2	" ferricyanide	($K_3Fe(CN)_6$ M/10–M/1,250)	–	Very faint to –	–	–	Very slow
3	" chromate	(K_2CrO_4 M/20–M/100)	–	Good	+		+
4	" bichromate	($K_2Cr_2O_7$ M/50–M/100)	–	Good	+		+
5	" permanganate	($KMnO_4$ M/50–M/200)	–	Bright	+	–	+
6	" hydroxide	(KOH M–M/6,250)	–	–	–	–	Very slow
7	" chlorate	($KClO_3$ M/10)	–	–	–	–	–
8	" persulfate	($K_2S_2O_8$ M/10–M/128)	–	–	–	–	–
9	" chromium alum	($Cr_2(SO_4)_3$. K_2SO_4 M/10)	–	Faint	Very slow	Very slow	–
10	Ferric ammonium alum	($Fe_2(SO_4)_3$. $(NH_4)_2SO_4$ M/10)	–	Faint	+		Very slow
10	" chloride	($FeCl_3$ M/10–M/250)	–	Fair	+		Slow
12	Ferrous sulfate	($FeSO_4$ M/10–M/6,250)	–	Fair	–	+	Slow
13	Copper sulfate	($CuSO_4$ M/5–M/125)	–	–	–	+	Very slow
14	Chromic acid	(CrO_3 M/100)	–	Bright	+		+
14	" sulfate	($Cr_2(SO_4)_3$ 2 per cent)	–	Faint	–	+	Slow
16	Chlorine water		–	–	+		+
17	Bromine "		–	–	+		+
18	Iodine in KI		–	–	+		+
19	Sodium hypochlorite	(Cl water + NaOH)	Faint flash	Bright	+		++
20	" hypobromite	(NaOBr, bromine water + NaOH)	Faint flash	Bright	+		++
21	" hypoiodite	(I in KI + NaOH)	–	Faint	+		+
22	Calcium hypochlorite	($Ca(OCl)_2$ saturated solution)	–	Good	+		++

23	Turnip juice	−	Bright	−	+	++
24	" " heated to 70°	−	Faint	−	+	Very slow
25	" " boiled	−	−	−	−	−
26	Albumin solution	−	−	−	−	−
27	" " + $KMnO_4$	−	Good	+	−	++
28	" " " " boiled 1 minute and filtered (no precipitate forms)	−	Good	+	−	++
29	Gelatin solution	−	−	−	−	−
30	" " + $KMnO_4$	−	Good	−	−	++
31	" " " " boiled 1 minute and filtered (no precipitate forms)	−	Good	+	−	++
32	Colloidal Ag	−	Bright	+		+
33	" Pt	−	Bright	+		+
34	" $Fe(OH)_3$ (dilute)	−	−	−	+	−
35	Sodium nucleoproteinate (liver)	−	−	−	+	−
36	" " (mammary gland)	−	−	−	−	−
37	" nucleate (yeast)	−	−	−	−	−
38	Squid blood (*Sepia esculenta*). Contains hemocyanin	−	Fair			++
39	" " " " boiled	−	Good			−
40	Lobster blood (*Palinurus japonicus*). Contains hemocyanin and tetronerythrin, a lipochrome	−	Faint			++
41	" blood (*Palinurus japonicus*) boiled	−	Fair			−
42	Annelid blood (*Laonome japonica*). Contains chlorocruorin	−	Good			
43	" " " " boiled	−	−			
44	Luminous pennatulid extract (*Cavernularia haberi*)	−	−	−	+	++
45	" ostracod " (*Cypridina hilgendorfii*)	−	−			+
46	" protozoan " (*Noctiluca miliaris*)	−	−	−	−	−
47	Firefly (*Luciola viticollis*) extract, luminous organs	−	−			++
48	Ferrous ferrocyanide ($Fe_2Fe(CN)_6$)	−	Faint	+		+
49	Zinc " ($Zn_2Fe(CN)_6$)	−	−	+		Very slow
50	Chromic oxide (Cr_2O_3)	−	−	−	−	Slow
51	" hydroxide ($Cr(OH)_3$)	−	−	−	Slow	+
52	Manganese dioxide (MnO_2)	−	Good	Slow	Slow	++

greater than the optimum. If that is the case, then lowering the temperature should reduce the reaction velocity to the optimum and light should appear. However, as we have seen, not lowering but raising the temperature causes luminescence with high oxygen concentration or high pyrogallol concentration.

I believe the explanation of these phenomena lies rather in another direction and that the effect of the temperature and concentration of reacting substances affects not only the reaction velocity but also the reaction products. While intensity of luminescence undoubtedly increases with increasing reaction velocity, the luminescence itself probably accompanies only one stage in the formation of a series of oxidation products. This stage is favored at a definite temperature and mass of reacting substances. Thus, in the oxidation of phosphorus several intermediate oxides are said to be formed. The oxidation takes place in steps and probably the luminescence is connected with only one of the steps in a chain of reactions. It is probable that a certain oxygen pressure and temperature favors that particular step at the expense of the others and so this oxygen concentration and temperature correspond to the optimum for luminescence.

The supposition that certain definite oxidation products of pyrogallol must be formed in order to produce light is borne out by the fact that pyrogallol must be oxidized in a particular way to obtain luminescence. The blackening of pyrogallol with absorption of oxygen in presence of alkali is a very well-known reaction, but luminescence does not accompany this type of oxidation. I have tried mixing all concentrations of pyrogallol and all concentrations of alkali in an endeavor to obtain some

light, but always with negative results. Likewise my attempts to obtain light during the electrolysis of salt solutions containing pyrogallol by means of the nascent oxygen at various kinds of anodes have met with negative results. A similar case is presented by luciferin which oxidizes spontaneously (most rapidly in presence of alkali) without light production and only produces light when oxidized in presence of luciferase.

To sum up the results of the dynamics of chemiluminescence we may say that certain oxyluminescences occur only if the substance is oxidized in a particular way under definite conditions of temperature and concentration and that this is probably due to a favoring of one step (with which the luminescence is associated) in a chain of oxidations. Providing temperature and concentration are such as to favor the step responsible for luminescence, then higher temperature and greater concentration result in increased intensity of luminescence.

Let us now turn to luminous organisms and consider the effect of temperature and of concentration of reacting substances (oxygen, luciferin and luciferase) on the luminescence. We have already seen that luminescence of a luciferin-luciferase mixture begins with an extraordinarily low oxygen tension and increases in intensity with increasing tension of oxygen, but that very soon an oxygen tension is reached where a maximum luminescence is obtained and further increase of oxygen tension gives no brighter light. In this respect the luminescence intensity —oxygen tension curve is no doubt very similar to the hæmoglobin saturation—oxygen tension curve. Hæmoglobin is about 50 per cent. saturated at 10 mm. oxygen pressure, 80 per cent. saturated at 20 mm. oxygen pressure

and completely saturated at pressures of oxygen well below the pressure of oxygen in air (152 mm. Hg). As the optimum oxygen tension for luminescence of luciferin is also well below that of air, mixtures of luciferin and luciferase luminesce with equal brilliancy whether air or pure oxygen is bubbled through them. To obtain an excess of oxygen it is only necessary to keep the solution saturated with air and statements regarding concentration of luciferin and luciferase and intensity or duration refer to excess of oxygen. Investigators who have studied the effect of increase in oxygen pressure on luminous animals have come to the same conclusions. High pressures of air or oxygen do not increase the intensity of luminescence (Dubois and Regnard, 1884).

The hydrogen ion concentration of crude solutions of luciferin and luciferase, made by extracting whole Cypridinas with hot or cold water is fairly constant, about $P_H = 9$, determined electrometrically. Such solutions have a high buffer value and the P_H does not change during oxidation of luciferin so that this variable is automatically controlled.

Because of difficulties in measuring low intensities of light which are constantly changing, no figures on light intensities can be given, but it is easy to establish the following facts: The greater the concentration of luciferin or luciferase the more intense the luminescence. The greater the concentration of luciferin the longer the duration of luminescence and the greater the concentration of luciferase, the shorter the luminescence lasts. Thus, if we mix concentrated luciferin and weak luciferase we get a bright light which lasts for a half hour or more, gradually growing more dim. Concentrated luciferase and weak

luciferin give a bright flash of light which disappears almost instantly. Concentrated luciferase and concentrated luciferin give a brilliant light which lasts for an intermediate length of time and weak luciferin and weak luciferase give a faint luminescence which lasts for an intermediate length of time.

These facts can all be explained by regarding luciferase as a catalyzer which accelerates the oxidation of luciferin and by assuming that intensity of luminescence is dependent on reaction velocity, *i.e.*, on rate of oxidation. Contrary to the condition for phosphorus and for pyrogallol there appears to be no optimum concentration of luciferase or luciferin, but the luminescence intensity increases gradually with increasing concentration of luminous substances up to the point where pure (?) luciferin and pure (?) luciferase, as secreted from the gland cells of the animal, come in contact with each other. This, the maximum brightness, is not to be compared with the light of an incandescent solid, but is nevertheless visible in a well-lighted room, out of direct sunlight.

The effect of temperature on *Cypridina* luminescence also bears out the preceding conclusions. For a given mixture of luciferin and luciferase the light becomes more intense with increasing temperature up to a definite optimum and then diminishes in intensity. The diminution in intensity above the optimum is due to a reversible change in the luciferase so that its active mass diminishes. This change becomes irreversible in the neighborhood of 70° (depending on various conditions), where coagulation of luciferase occurs. Light will appear at 0° but it is far less intense than light at higher temperatures and it is more yellow in color. The light of optimum temperatures

is quite blue. The weaker light at temperatures above the optimum is also more yellow in color. I believe this difference in color is a function of the slowed reaction velocity, for a mixture of luciferin and luciferase which gives a bluish luminescence at room temperature, will give a weaker and yellowish luminescence if diluted with water. Dilution with water will slow the reaction velocity. If the difference in color were not real but due to change in color sensitivity of the eye with different intensities of such relatively weak light (Purkinje phenomenon), the weaker light should appear more blue. As the weaker light appears more yellow, I therefore believe the color difference is actual and not subjective.

A minimum, optimum, and maximum temperature for luminescence is observed in all luminous organisms. The minimum is usually very low. Luminous bacteria will still light at –11.5° C. The power to luminesce under ordinary conditions is not destroyed by exposure to liquid air, for, on raising the temperature, light again appears (Macfayden, 1900, 1902). Almost all organisms will luminesce at 0° C., and the luminescence minimum probably represents the point at which complete freezing of the luminous solution occurs. It is very low with bacteria because they are solutions in capillary spaces of very small size, a condition tending to lower the freezing point.

The luminescence maximum represents the point at which luciferase is reversibly changed so as to be no longer active. If the temperature is again lowered the luciferase again becomes active and light reappears. Some degrees above this, and in all forms well below the boiling point, luciferase is coagulated and destroyed. As the coagulation point of proteins depends on many

factors, such as time of heating, salt content, acidity, etc., so the luciferases of different animals coagulate at different temperatures depending on these conditions. Some of the more reliable observations on these critical temperatures are collected in Table 14.

We are thus led to the conclusion that intensity of luminescence is dependent on the velocity of oxidation of luciferin and that with lowered reaction velocity the spectral composition of the light changes. The maximum emission shifts toward the yellow. I believe, however, that in *Cypridina* also, the luminescence intensity depends not only on reaction velocity but on the particular manner in which luciferin is oxidized. *Cypridina* luciferin will luminesce only in presence of *Cypridina* luciferase and no light can be obtained from *Cypridina* luciferin and a host of different oxidizers (with or without H_2O_2) such as are able to oxidize pyrogallol. Luciferin will also oxidize in the air spontaneously but no light is produced. It is easy to show that this spontaneous oxidation may be much more rapid than an oxidation with luciferase and yet light appear only in presence of the latter. If a concentrated solution of luciferin is kept near the boiling point it will be completely oxidized to oxyluciferin in four or five minutes. No light appears if air or even if pure oxygen is bubbled through it. The same solution kept at 20° with a small amount of luciferase will luminesce continuously and not be completely oxidized to oxyluciferin in a half hour. We can, however, cause the luciferin to oxidize as rapidly at 20° by adding concentrated luciferase as does the luciferin near the boiling point without luciferase. A bright light is produced in the former case, none in the latter case. The oxyluciferin

TABLE 14

Temperature Limits of Luminescence for Luminous Organisms

Organism	Author and date	Minimum	Optimum	Maximum
Pseudomonas javanica	Eijkman, 1892	−20°	25–33°	45°
Bacterium phosphorescens	Lehmann, 1889	−12°	.....	39.5°
Bacterium phosphoreum	Molish, 1904, book	−5°	16–18°	28°
Light bacteria	Tarchanoff, 1902	−7°	15–25°	37°
Light bacteria	Harvey, E. N., 1913	−11.5	15–20°	38°
Mycelium X	Molish, 1904	.....	15–25°	36°
Lampyrids	Macaire, 1821	−10	33°	46–50°
Pyrophorus noctilucus	Dubois, 1886	.....	20–25°	47°
Photuris pennsylvanica	Lund, 1911	.....	.....	50°
Luciola viticollis	Harvey, E. B., 1915	<0°	.....	42°
Cypridina hilgendorfii	Harvey, E. N., 1915	<0°	.....	52–54°
Cyclopina gracilis	Lund, 1911	.....	.....	50°
Phyllirrhoë bucephalum	Panceri, 1872	44°	.....	61°
Pyrosoma	Panceri, 1872	<0°	.....	60°
Mnemiopsis Leidyi	Peters, 1905	9°	21°	37°
Noctiluca miliaris	Quatrefages, 1850	1°	.....	40°
Noctiluca miliaris	Harvey, E. B., 1917	<0°	.....	48°
Cavernularia haberi	Harvey, E. N., 1915	<0°	.....	52°
Watasenia scintillans	Shoji, R, 1919	.....	16–31°	49°

formed from spontaneous oxidation of luciferin appears to be the same as that formed with luciferase present. Both give luciferin again on reduction. Perhaps the reaction takes place in two stages, similar to those supposed to occur in other enzyme actions:

luciferin + luciferase = luciferinluciferase

luciferinluciferase + O (or minus H_2) = oxyluciferin + luciferase.

We may then assume as a tentative hypothesis that luminescence only occurs during oxidation (addition of O or removal of H) of the luciferinluciferase compound.

We have just seen that the effect of cooling a *Cypridina* extract containing luciferin and luciferase and luminescing with a bluish light, is to reduce the intensity and change the shade toward the yellow. Velocity of oxidation must be lowered and with the same concentration of luciferase lowered velocity means more light of the longer wave-lengths. A very instructive experiment on color of the light can be carried out with animals having different colored lights and so closely related that their luciferins and luciferases will interact with each other. Such a case is presented by the American fireflies, *Photinus* and *Photuris*. *Photinus* emits an orange light, while *Photuris* emits a greenish yellow light. The difference in color is especially noticeable when the luminous organs of the two forms are ground up in separate mortars. As shown by Coblentz, the difference in color is real, the spectrum of *Photinus* extending farther into the red than that of *Photuris* (see Fig. 8). We can easily prepare luciferin and luciferase from the two fireflies and make the following mixtures:

Photinus luciferin × *Photinus* luciferase = reddish light.

Photinus luciferin × *Photuris* luciferase = yellowish light.

Photuris luciferin × *Photuris* luciferase = yellowish light.

Photuris luciferin × *Photinus* luciferase = reddish light.

Thus the color of the light in these "crosses" is that characteristic of the animal supplying the luciferase. To bring this fact in line with what we have already said regarding reaction velocity and luminescence, we must believe that the *Photinus* luciferase oxidizes at a slower rate than the *Photuris* luciferase. In this connection it is of interest to recall that the *Photuris* light as emitted by the insect becomes reddish at high temperatures, or if the insect is plunged into alcohol, both conditions which bring about partial coagulation of the luciferase and reduce its active mass.

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A few of the enormous number of papers on luminescence are included in the list below. The attempt is made to list only those dealing with the structure, chemistry or physiology of luminous animals and the physical nature of their light, together with a small number of general interest. More complete works on light and luminescence come first and original articles follow Authors' names are arranged alphabetically, their papers chronologically. A fairly complete list of literature covering the whole field of Bioluminescence is given by Mangold, 1910. The 1913 paper of Dubois gives a bibliography of his own contributions up to this date so that only those papers to which special reference is made are included below.

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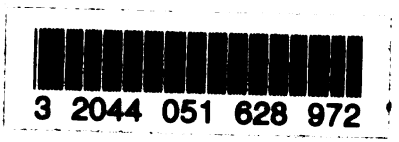

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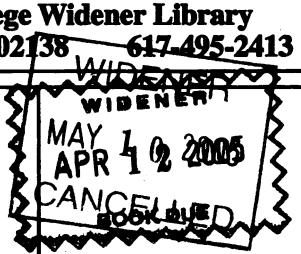

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